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The Rate of Progression of Poliomyelitis Virus in Nerves. DAVID BODIAN AND HOWARD A. HOWE 79

The Effect of Spinal Transection on the Spread of Poliomyelitis Virus in the Nervous System of the Rhesus Monkey. HOWARD A. HOWE AND DAVID BODIAN 86

Refractoriness of Nerve Cells to Poliomyelitis Virus After Interruption of Their Axones. HOWARD A. HOWE AND DAVID BODIAN 92

The Pathology of Early Arrested and Non-paralytic Poliomyelitis. DAVID BODIAN AND HOWARD A. HOWE 135

Poliomyelitis in the Chimpanzee: A Clinical-pathological Study. HOWARD A. HOWE AND DAVID BODIAN 149

Neuropathological Evidence on the Portal of Entry Problem in Human Poliomyelitis. HOWARD A. HOWE AND DAVID BODIAN 183

A New Treatment for Burns. KENNETH L. PICKRELL, M.D. . 217

Book Reviews 222

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THE RATE OF PROGRESSION OF POLIOMYELITIS VIRUS IN NERVES[1]

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Submitted for publication June 2, 1941

There exist at the present time no estimates of migration velocities of neurotropic viruses in nerves which can in any sense be considered accurate. (See Doerr, '39, for review of subject.) Accurate data on this point would be of great value not only in the enlargement of our understanding of virus-host processes, but also in the attack on the larger problems concerned in the movements of biological substances in protoplasm, and particularly in axoplasm. For this purpose the neurotropic virus of poliomyelitis possesses both advantages and disadvantages. The advantages are the remarkable selectivity of this virus for neurones (Howe and Bodian, '41, Bodian and Howe, '40a and '41) and the apparently negligible disturbance of axonal function occasioned by its movement within nerves (O'Leary, Heinbecker, and Bishop, '32). Obvious disadvantages are the inaccuracies arising from the inability to assay the virus chemically, and the many variables introduced when two variable biological systems, the virus and the host, are combined and interact.

One of the chief variables in this respect is that introduced by differences in the incubation period, since under identical conditions of inoculation by either the intracerebral or intraneural route, any given initial sign of poliomyelitis (fever spike or paralysis) may vary in its onset in different animals by as much as 1 to 3 days, or more. This variable, which is largely introduced in the central nervous system, can be reduced in importance by using the method of Hurst ('30), namely, inoculation and subsequent cutting of a nerve at a higher level.

[1] This investigation was made possible by a grant from the Commonwealth Fund.

By this method it is possible to determine how soon after inoculation the nerve must be cut to prevent an infective dose from passing the point of section and thus infecting the spinal cord. Hurst merely states that the sciatic nerve must be cut 24 hours after inoculation, at a distance centrally which he does not specify, to prevent the virus from passing to the spinal cord, but apparently he did not attempt to estimate the rate of progression of the virus. When the immersion method of inoculation is used (Bodian and Howe, '40b and '41), as described below, the variables introduced by inoculation with the injection method can further be controlled, since the method produces consistent "takes" in normal animals, does not involve any injection pressure, and thus permits "inoculation" of the nerve fibers in the nerve at a single point. The only time factor in addition to time of progression of virus which must be considered with this method is the latent period between the time of inoculation and the time of beginning spread in the nerve (Sabin, '37).

The method used and the results obtained can be described briefly:

The right sciatic nerve (tibial and peroneal) of rhesus monkeys was sectioned at the lower thigh level with sharp scissors and the central cut end of the nerve immersed in 0.15 cc. of a homogeneous and potent sample of cord suspension of Rockefeller MV virus for 5 minutes, after which the excess suspension was removed with a cotton-wool sponge. All animals were inoculated with virus-containing material from the same sample, and the time of inoculation was taken as the moment of initial contact of exposed nerve fibers with virus. Two series of animals were so treated. In the first series of 8 animals (Table I), except for a control animal, the nerve was re-exposed and frozen across with CO_2 ice at a distance of 15 mm. proximal to the severed end. This was done in different animals at intervals of 5, 8, 11, 14, 17, 20 and 23 hours after inoculation, to determine the duration of the latent period plus the time required for the virus to traverse the initial 15 mm. of nerve. The nerve was frozen instead of being sectioned in order to prevent re-infection of the cut end by virus which might persist in the field. In the second series of 7 animals (Table II) the nerve was re-exposed at a greater distance above the point of inoculation and sectioned. This was done at intervals of 24, 30, and 40 hours in each of 2 animals. A control animal in this series was inoculated with the others, but his sciatic nerve was not subsequently transected. All animals were allowed to survive at least 3 weeks and if signs of poliomyelitis did not develop by that time, they were killed and perfused with fixing fluid (10 per cent formol and 1 per cent acetic acid). The segment of sciatic nerve between the site of inoculation and the point of subsequent transection in series B was then carefully measured. Because of the retraction of the cut ends

at the time of inoculation, the distances between the points of inoculation and points of subsequent transection in this series were made as long as possible, to reduce the error from this source. Tables I and II present the pertinent data obtained.

Table I shows that virus failed to traverse the distance of 15 mm. in any time interval less than 17 hours in this series of animals. Ani-

TABLE I

ANIMAL	INTERVAL BETWEEN INOCULATION AND FREEZE AT DISTANCE OF 15 MM. PROXIMAL TO INOCULATION	POLIOMYELITIC PARALYSIS
A220	5 hours	None
A222	8 hours	None
A226	11 hours	None
A225	14 hours	None
A224	17 hours	14 days
A223	20 hours	8 days
A221	23 hours	None. Secondary infection
A227	Control	6 days

TABLE II

CASE NUMBER	INTERVAL BETWEEN INOCULATION AND TRANSECTION (i)	LENGTH OF NERVE BETWEEN INOCULATION SITE AND TRANSECTION (d)	$\frac{d}{i}$	POLIOMYELITIC PARALYSIS
A189	24 hours	53 mm.	2.20	None
A190	24 hours	78 mm.	3.25	None
A193	30 hours	90 mm.	3.00	None
A194	30 hours	67 mm.	2.23	None
A196	40 hours	70 mm.	1.75	5 days
A197	40 hours	80 mm.	2.00	None
A198	Control			5 days

mal A221, which had experienced freezing of the nerve 23 hours after inoculation, developed a suppurative secondary infection of his wound on the day following the second exposure of the nerve. This may account for the failure of development of poliomyelitis in this case. It is noteworthy that although the control animal was paralyzed after 6 days, which is a typical incubation period for inoculation by this method (Bodian and Howe, '40b), the animals in the experimental

group which became paralyzed had incubation periods of longer duration. This suggests either that the procedure of freezing altered the metabolism of the neurones involved sufficiently to affect the incubation period, or that the freezing prevented as large a dose from passing up the nerve as in the control case. The latter supposition is supported by the fact that the animal which had the sciatic nerve frozen at 17 hours after inoculation (A224) had a much longer incubation period than the animal which had the nerve frozen at 20 hours. The extremely long incubation period of the former animal (14 days) suggests that the period of 17 hours is close to the threshold period for spread of an infective dose of the virus along 15 mm. of nerve, under the conditions of this experiment and including the latent period which may exist before spread begins.

Table II shows the results of the second series of experiments, in which the determination of the time of spread along a longer segment of nerve was attempted, in order to rule out some of the inaccuracies which might result from the use of short segments of nerve. In this series the nerve was cut at distances 53 to 90 mm. proximal to the point of inoculation. This distance was far enough to preclude any chance of reinfection of the second cut end with virus. Only one animal, other than the control animal, became paralyzed. It can be seen that in this animal the ratio between length of nerve traversed and time allowed for spread over this distance is less than in any of the other animals. Since in three of the animals which failed to become paralyzed (A197, A189, and A194) this ratio is greater, but by a relatively small amount, it may be assumed that the ratio for animal A196 represents a figure close to the threshold for a minimal infective dose.

This ratio of 1.75 mm. per hour, however, is not corrected for any possible latent period which might exist preceding the beginning spread of the virus. When this is taken into account, a fair approximation of the rate of spread of a minimal infective dose of virus can be expressed as follows, assuming a uniform rate:

$$\text{Rate}_{\text{m.i.d.}} = \frac{\text{Length of nerve segment}}{\text{Time interval} - \text{Latent period}}$$

or

$$R = \frac{d}{t - 1}$$

The rate is the minimal rate necessary for an infective dose to pass the proximal end of the nerve segment, in any animal which becomes infected. In case A196, the rate can be estimated with a fair degree of accuracy by using the data obtained in Series 1. Thus, assuming the time required to traverse 15 mm. of nerve, plus the duration of the latent period, to be 17 hours, the rate of spread of virus in case A196 can be calculated as follows:

$$R = \frac{d - 15}{t - 17} = \frac{70 - 15}{40 - 17} = 2.4 \text{ mm. per hour}$$

If this rate of spread is approximately correct, one can, by deducting the time necessary for spread along 15 mm. of nerve (6 hours) estimate the latent period as 17 hours minus this time, or 11 hours. That this latent period of approximately 11 hours does not represent a period of quiescence of the virus is probable. A more likely supposition is that it also includes a variable period during which an infectious quantity of virus is making its way into the severed ends of the nerve fibers. This time interval may conceivably be dependent on the concentration of the virus suspension used, the method used for suspending the virus, and upon other variables.

DISCUSSION

Evidence for the spread of poliomyelitis virus within the axoplasm of peripheral nerve fibers has been presented in a previous report (Bodian and Howe, '41), in which the mechanism of spread of virus in the nerve was discussed. It is obvious that the data on the rate of spread presented here represent only rough estimates, rather than accurate measurements, although some of the principle variables, such as the latent period preceding spread, the incubation period in the central nervous system, the homogeneity of the infectious material, and the mode of inoculation, have been eliminated or have been fairly well controlled.

The use of peripheral nerves, instead of the cerebrospinal axis, for determination of the rate of spread of virus, is made necessary because of the fact that in the central nervous system the uniform spread of the virus in axons is prevented by spread along collateral pathways, interruption of spread in synaptic centers, growth of the virus in the synaptic centers resulting in changes in concentration of the virus, and

pathological reactions in the gray centers which may introduce disturbing factors. Although it is clear that the spread of virus along the cerebrospinal axis may be extraordinarily rapid, the factors just mentioned are probably responsible for the great variability observed in this spread. In peripheral nerves, on the contrary, these factors are either non-operative or of negligible importance as far as we know.

Koppisch ('35) has found that after inoculation of herpes virus on the rabbit's cornea, the virus can first be detected in the corresponding Gasserian ganglion after 48 hours. Estimating the distance traversed along the ciliary and ophthalmic nerves as 16.6 mm., Koppisch concludes that the rate of spread is about 0.35 mm. per hour, but since the latent period before spread begins was not considered, this figure is probably considerably less than the actual rate. After inoculation into muscle, Sabin found that the initial latent period, before spread of virus in the corresponding nerve began, was about 24 to 30 hours for pseudorabies and 48 to 72 hours for B virus. This compares with about 11 hours for poliomyelitis virus under quite different experimental conditions. Obviously then, Koppisch's figure for the rate of spread of herpes virus in peripheral nerve cannot be accepted without correction for the latent period.

It is also interesting to compare the rate of spread of virus in nerve with the rate of spread of diffusible dyes, such as methylene blue (Perdrau, '37). Perdrau gives as the lowest figure for spread in mammalian sciatic nerve, about 4 to 5 cm. in 4 hours, a considerably more rapid rate than we have found for poliomyelitis virus (2.4 mm. per hour). The fact that Perdrau found that colloidal solutions do not spread along the sciatic nerve, indicates that the colloidal protein particles of the virus are only enabled to penetrate and spread in the axis-cylinder by virtue of a specific adaptability to the physicochemical conditions existing in the neuroplasm of the host.

SUMMARY

1. The rate of progression of poliomyelitis virus in the sciatic nerve of the rhesus monkey was estimated to be approximately 2.4 mm. per hour. The method used was inoculation at a single point in the nerve and subsequent cutting of the nerve at a higher level, at various intervals in different animals, to prevent an infective dose of virus from

reaching the spinal cord. An attempt was made thus to control the variability introduced by the incubation period. Also controlled were the method of inoculation, the homogeneity of the virus suspension, and the latent period before spread begins.

2. The latent period was estimated to be about 11 hours.

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THE EFFECT OF SPINAL TRANSECTION ON THE SPREAD OF POLIOMYELITIS VIRUS IN THE NERVOUS SYSTEM OF THE RHESUS MONKEY[1]

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Submitted for publication July 7, 1941

Experiments frequently cited as proof of the neuronal conduction of poliomyelitis virus are those of Jungeblut and Spring ('30) and of Brodie and Elvidge ('34), which purported to show that virus did not cross a transection of the spinal cord. The total number of experiments performed by the last two workers is unspecified, but the much quoted conclusion of Jungeblut and Spring was based upon two animals, one of which furnished no data which could be interpreted by the authors. Toomey ('34b), on the other hand, reported that out of four animals with spinal transections, virus could be recovered beyond the point of transection in two, although typical lesions were not found in this area. He postulated that virus descended along the paravertebral sympathetic chains and then reentered the spinal cord below the point of transection. A critical examination of Toomey's experiments shows that in his animals poliomyelitis was induced by intracerebral inoculation at the time of spinal transection. Since it is well known that intracerebral inoculation produces wide dissemination of the virus in the cerebrospinal fluid (Schaeffer and Muckenfuss, '38) and that artificial breaks in the pia greatly facilitate takes (Howe and Peele, '39), there is no reason to rule out the cerebrospinal fluid as the medium of virus transport to the lumbar cord in these experiments.

In an effort critically to reëxamine the question of conduction across or around a transection of the spinal cord, 18 rhesus monkeys were

[1] Supported by a grant from the Commonwealth Fund.

[2] On leave of absence from the Department of Anatomy, Western Reserve University, Cleveland, Ohio.

studied. These were subjected to transection of the cord at the 7th to 9th thoracic levels. The experiments fall into three groups, in which the procedures were varied slightly in order to answer questions

TABLE I

Progression of Virus in Spinal Rhesus Monkeys

EXPERIMENT	INTERVAL FROM TRANSECTION TO INOCULATION	MODE OF INOCULATION	PARALYSIS		OTHER PROCEDURES
			Above transection	Below transection	
	days				
I 739	6	Intranasal	+	0	L.* cord neg. for virus
740	6	Intranasal	+	0	L. cord neg. for virus
732	10	Intracereb.	+	0	
748	0	Subdural space over hemisphere			L. cord pos. for virus
	16	Intranasal	+	+	
II 815	6	Intrasciatic	+	+	
814	6	Intrasciatic			
	15	L.* cord stab†	+	+	
813	6	Intrasciatic			
	15	L. cord stab	0	+	
812	6	Intrasciatic			
	15	L. cord stab	0	+	
III 987	13	L. cord stab	0	+	
A93	12	L. cord stab	0	+	
A96	15	L. cord stab	0	+	
952	14	L. cord stab	+	+	
971	13	L. cord stab	+	+	
989	12	L. cord stab	+	+	
A98	14	L. cord stab	+	+	
A94	14	L. cord stab	+	+	Sympathectomy
A97	12	L. cord stab	+	+	Sympathectomy
A99	14	L. cord stab	+	+	Sympathectomy
A108	11	L. cord stab	+	+	Sympathectomy Double ligation of spinal dura

* L. indicates lumbar.

† Paralysis due to intrasciatic inoculation.

as they arose during the progress of the work. The first group consisted of four animals (Table I) which were inoculated intranasally or intracerebrally at various intervals following cord transection.

Three of these animals (739, 740, and 732) contracted typical poliomyelitis, as shown by paralysis of the arms and lesions in the cervical cord, but neither virus nor lesions could be demonstrated in the lumbar cord. The fourth animal of this series (748) was inoculated at the time of cord transection, whereas in the others of this group 6 to 10 days had elapsed between these two procedures. The method of inoculation consisted of opening a large dural flap over one hemisphere and allowing 0.75 cc. of 20 per cent MV virus to spread over the cortex. The dura was then carefully reapproximated. It had been previously shown that this procedure was harmless unless injuries were produced in the pia (Howe and Peele, '39). The animal showed no reaction for 16 days, after which time he was reinoculated intranasally on two successive days. Two days following the last intranasal inoculation the temperature rose sharply and two days later the arms were paralysed. During the next two days the arms became completely flaccid and the animal was killed *in extremis*. Injection of india ink into the cisterna magna while fluid was removed from the lumbar sac, produced no evidence of continuity of the subarachnoid space across the region of transection. Sections of the cervical cord revealed severe poliomyelitic lesions, while the findings were somewhat equivocal in the lumbar cord because of concomitant atrophic changes. However, upon intracerebral inoculation of the lumbar cord into a test animal typical poliomyelitis resulted.

The results obtained in animal 748, then, suggested either that the virus had reached the lumbar cord within a few hours after the initial inoculation and before blockage of cerebrospinal fluid had taken place, or else the virus had found some other method of circumventing the transection of the cord. A serious difficulty which was encountered in these experiments, by us and also by the others who had also attempted them, was the early death of the animals after paralysis of the arms. It was thought that the 24 to 48 hours which elapsed between paralysis of the arms, and exitus, were not sufficient for the passage of virus by any devious route around a cord transection. Accordingly in the next series of animals (series II, Table I) the procedure was revised: the cord was transected; 6 days were allowed for scar formation; and the right sciatic nerve was inoculated with 1 cc. of 25 per cent MV virus. On the seventh day following inoculation

one animal (815) developed paralysis of the arms and died the following day. Another animal (814) which had shown only fever up to the 9th day was reinoculated by direct stab into the lumbar cord. The following day it had paralysis of the arms, which had doubtless been the result of the first inoculation rather than that of the previous day. Two other animals, 812 and 813, which were treated in the fashion of 814 showed loss of leg reflexes and typical lesions in the lumbar region following inoculation of the cord but did not exhibit clinical or microscopic evidence of disease in the cervical cord. No animal showed continuity of nerve tissue or subarachnoid space across the transection.

The series of animals just mentioned was very similar to one described by Toomey ('34a) and suggested that in some cases it was possible for virus to circumvent a spinal transection by ascending in the paravertebral sympathetic chains, having gained access to them either from the cord or sciatic nerve. Since the sciatic connection with the sympathetic chains seemed the more likely possibility it was eliminated in the next series of experiments. In this series a group of eleven animals was prepared with spinal transections at the level of Th 8–9 (Group III, Table I). To facilitate scarring, a piece of muscle was placed between the cut surfaces of the cord, and 11 to 15 days were allowed to elapse before inoculation of 0.1–0.4 cc. of MV virus directly into the lumbar cord. At death this scar was found to be impermeable to india ink or methylene blue introduced into the subarachnoid space. In addition, four of these animals were subjected to bilateral sympathectomy of 2–3 ganglia at the level of transection. As may be seen in Table I every one of the sympathectomized animals contracted paralysis above the region of transection and 4 of the 7 unsympathectomized animals fared similarly. It is also to be emphasized that in animal A108 which was sympathectomized and subsequently contracted paralysis of the arms, a segment 2 cm. long was removed from the spinal cord. At this point the dura was sutured, freed anteriorly from the spinal canal and doubly ligated in the region from which the cord segment was removed. In this case, therefore, there was no possibility that the virus passed directly from lumbar to cervical cord.

It was then perfectly clear that interruption of the continuity of the spinal cord, the sympathetic nervous system, and the spinal sub-

arachnoid space did not prevent virus from passing into the rostral portion of the neuraxis. The only possible remaining routes for virus were lymphatics, blood stream, or some unusual nervous connections. Certain facts favored the last of these possibilities, and indicated the participation of the vagus nerve in the sympathectomized cases and in two of the unsympathectomized ones. Four of the animals in particular (two sympathectomized) showed an unusual clinical picture which suggested a vagus syndrome. This consisted of sudden syncope or death without previous involvement of the arms as would be expected in an ordinary ascending infection. Examination of the brains of these animals in serial sections showed unusually heavy involvement of the vagal nuclei in the medulla. In two of the brains the lesions were largely confined to these centers. Examination of the brains of the remaining sympathectomized animals which succumbed to generalized paralysis without showing this particular clinical syndrome, showed a more generalized spread of virus in the medulla but there was nevertheless involvement of the vagal centers in each case. The brains of the two unsympathectomized animals which also were paralysed in the arms showed no lesions in the nuclei of the tenth nerve.

It is thus indicated that virus may pass from the cord via the sympathetic fibers to the paravertebral chains and after ascending in them, reenter the cord; or in the absence of intact sympathetic chains it may continue to the walls of the gut, where in Meissner's and Auerbach's plexuses it comes into contact with the preganglionic fibers of the vagus and ascends to the brain. The crucial demonstration of this latter deduction requires transection of the cord together with bilateral sympathectomy and vagotomy. If time permits the carrying out of such complicated procedures these experiments will be reported in greater detail, since they might shed considerable light upon the potentialities for the movement of virus in the peripheral nervous system.

SUMMARY

1. The movements of poliomyelitis virus were studied in the central nervous systems of three groups of rhesus monkeys after transection of the spinal cord in the mid-thoracic region.

2. The first group consisted of four animals in which paralysis was

induced by intracerebral or intranasal inoculation. Virus and lesions of poliomyelitis were demonstrated in one case in the lumbar cord.

3. The second group contained four animals in which the sciatic nerve was inoculated. Two of these showed progression of virus into the cord above the level of transection—possibly along the paravertebral sympathetic chains.

4. The third group consisted of eleven animals which were inoculated directly into the lumbar cord. Four of these had bilateral sympathectomies of 2 to 3 ganglia at the level of spinal cord transection. Eight of this third group of eleven spinal animals showed progression of virus into the balance of the neuraxis. In all the sympathectomized animals and two of the others there was clinical and pathological evidence pointing to a pathway for virus from the spinal cord to the wall of the gut via sympathetic nerve fibers and thence to the brain stem along the vagus nerve.

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REFRACTORINESS OF NERVE CELLS TO POLIOMYELITIS VIRUS AFTER INTERRUPTION OF THEIR AXONES[1]

HOWARD A. HOWE AND DAVID BODIAN[2]

From the Department of Anatomy, The Johns Hopkins University

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If one thinks of each cell in the body as a discrete organism within a very complex and constantly changing environment it becomes apparent that "immunity" in the narrow sense describes only a small number of the multiple reactions of the cell which make for its resistance to destructive outside influences. In addition to immune responses resulting from direct or indirect exposure to an infective agent, there are many unexplained phases of the constantly changing equilibrium of the cell and its environment which operate as generalized protective reactions. For example, it has been shown that resistance of mice to the neurotropic viruses of equine encephalomyelitis and vesicular stomatitis is conditioned by certain general factors such as growth and diet which appear to affect the progression of these viruses from non-nervous tissues into the central nervous system (1–2). Metabolic mechanisms of this type, however definite their effects, are difficult to analyse because of their extremely general nature. The most favorable conditions for the study of the effects of metabolic changes upon the ability of cells to resist infectious agents would be found in a restricted group of cells markedly susceptible to a very specific infection. These conditions are met with in the cells of the central nervous system which are attacked by the highly neurotropic virus of poliomyelitis, and the present study will show that the resistance of these nerve cells to destruction by poliomyelitis virus is strikingly increased by the metabolic changes in the cell body which accompany the regeneration of the axone. The advantage of such a

[1] Supported by a grant from the Commonwealth Fund.

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situation is obvious—opportunity is afforded to study the entire cycle of changes which occur in given groups of nerve cells during all the phases of axonal regeneration with special reference to the conditions which make the cells favorable hosts for poliomyelitis virus. Ideal for this purpose are two groups which under ordinary conditions are highly susceptible to destruction by poliomyelitis virus, the mitral cells of the olfactory bulb and the somatic motor neurones of the spinal cord. (For preliminary reports see references 3 and 4.) These cells were stimulated to regenerative activity by section of their axones, the olfactory tract in the one case and the sciatic nerve or entire roots in the other.

MATERIAL AND METHODS

The investigation was carried out on rhesus monkeys which were subsequently infected, unless otherwise noted, with the Rockefeller MV strain of poliomyelitis virus. The experiments were terminated in all cases by etherization and bleeding of the animals, and perfusion through the heart with 200 cc. of normal saline followed by 1000 cc. of 10 per cent formol containing 1 per cent acetic acid. Paraffin sections at 10 micra were routinely prepared by the gallocyanin method of Einarson (6).

THE MITRAL CELLS OF THE OLFACTORY BULB

Refractoriness of the nerve cell following section of its axone was first observed in the olfactory bulb. It has previously been reported that following section of both olfactory tracts the olfactory bulbs were still susceptible to virus invasion after intranasal inoculation (5). Under these conditions animals developed fever at the same time as unoperated controls but showed no indication of paralysis, although the controls invariably became prostrate. Microscopic examination of the olfactory bulbs and the remainder of the brain revealed typical lesions in the former but no sign of invasion in the latter. These observations were reported in preliminary form, but since that time the series has been enlarged to include thirty-three animals. It soon became apparent in a larger number of experiments, that there was considerable variability in the response of the animals. Some developed very little if any fever and showed few lesions in the olfactory

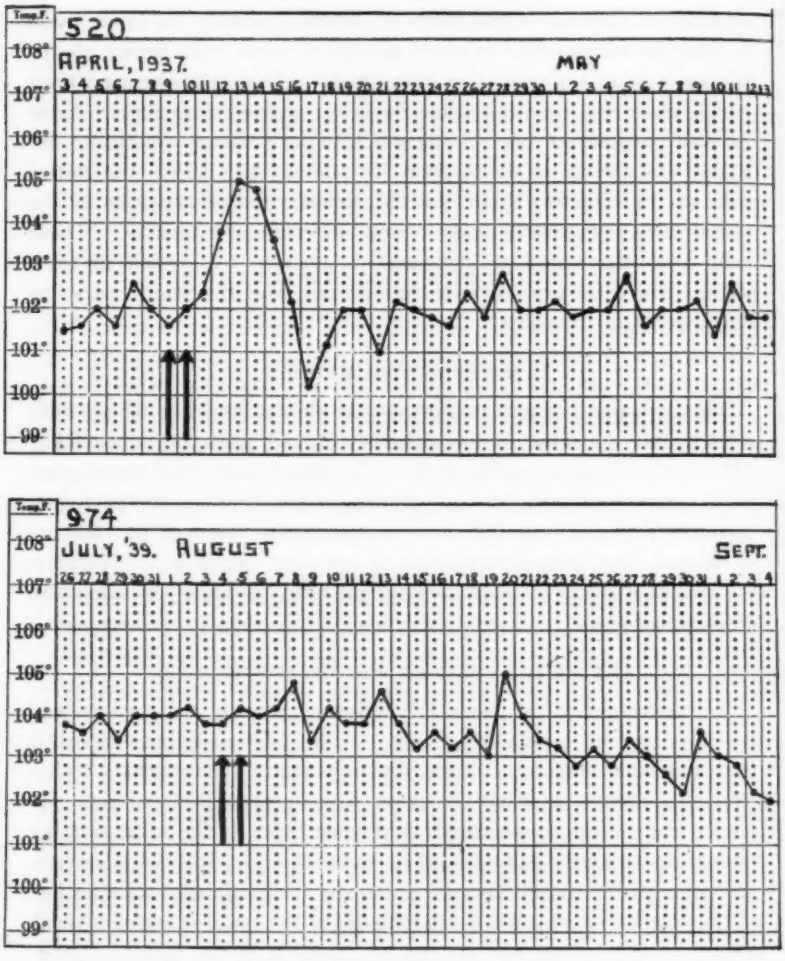

CHART 1

bulbs. This almost completely refractory state seemed to be correlated with the length of time which had elapsed after the section of the olfactory tracts. Chart 1 shows a comparison between the temperature

charts of two animals which were inoculated intranasally respectively 4 days (520) and 3 months (974) following olfactory tract section. It is quite apparent that in the latter the febrile reaction was abolished. Since none of the animals in this series became paralysed and the majority were used for another study, their bulbs did not come under observation during the acute stages of the disease.

In order critically to test this observation, 18 experiments were set up in which either only one olfactory tract was sectioned or a time interval allowed to elapse between each tract section. Two animals were killed 5 and 7 months after operation without inoculation. The remainder were inoculated intranasally with MV virus on two successive days, 0.5 cc. being dripped into each nostril with the head inverted. The experiment was terminated at the time of paralysis in the case of unilateral tract section, or when the controls became paralysed in the case of bilateral tract section.

In each of the control animals the olfactory bulb of the operated side showed the same picture, and the character of the histological changes is indicated in the first pair of photographs in plate I. The normal bulb is represented by figure 1. On the side of the tract section (fig. 2) there are nearly as many mitral cells as on the control side but their size is considerably smaller than normal. In addition to this the cells have undergone chromatolysis—that is, the quantity of Nissl substance in the cytoplasm is diminished (Plate II, figs. 1 and 2). These changes in the mitral cells are the direct result of the cutting of their axones as they pass in the olfactory tract from the olfactory bulb to the secondary olfactory nuclei in the brain. Comparison with preparations made within two weeks of tract section indicates that this reaction probably reaches a maximum within this early period and that subsequently an equilibrium is reached in which the cells persist indefinitely at reduced size. The gross size of the bulb is considerably reduced as the result of long standing isolation from the rest of the central nervous system by fiber loss as well as cell shrinkage.

Table I shows the result in 5 cases in which 2 to 15 days prior to inoculation, the olfactory tract was cut approximately midway between the olfactory bulb and the olfactory trigone. Inoculation was performed by dripping virus into the nostrils with the head inverted.

With the exception of the first case (522) in which virus probably did not reach the bulb, there was a progressive diminution of reaction to virus in the bulbs as a longer interval elapsed between tract section and inoculation. The second pair of photographs (Plate III), taken from animal 909, shows the lesions of poliomyelitis in the normal olfactory bulb (fig. 1) in comparison with the bulb (fig. 2) which had suffered olfactory tract section two days before the animal received an intranasal inoculation. There is relatively little difference in the amount of invasion on the two sides. As a reaction to the generalized encephalitis of the animal there is a marked meningeal infiltration of cells, largely lymphocytes, which collect most densely around pial

TABLE I

*Unilateral olfactory tract sections—virus dripped into nose**

EXPERIMENT	OPERATION	LESIONS OF POLIOMYELITIS	
522	Rt tract cut 2 days	Rt bulb 0	Lt bulb ++++
909	Rt tract cut 2 days	Rt bulb +++	Lt bulb ++++
922	Lt tract cut 7 days	Lt bulb ++	Rt bulb ++++
637	Lt tract cut 8 days	Lt bulb +	Rt bulb ++++
898	Lt tract cut 15 days	Lt bulb +	Rt bulb ++++

* Since lymphocytic infiltrations in the meninges are common to the pial surfaces of all basal portions of the brain, only pathological change in the bulb itself is considered as positive evidence of virus invasion.

blood vessels. At the same time the normal architecture of the bulb is obliterated by the increase in the phagocytic elements of the supporting tissues which follows upon the destruction of the susceptible mitral cells by the virus. There is also lymphocytic cuffing of smaller vessels throughout all portions of the bulbs. (See reference 7, for evidence that these cellular reactions are the result of specific interaction between nerve cells and virus.) A contrast to this picture is that seen in the pair of bulbs from animal 898 in which the left tract had been cut 15 days prior to inoculation (Plate IV). As in the other case, the normal bulb (fig. 1) is maximally invaded with almost complete destruction of its mitral cells. The operated bulb (fig. 2) shows the reaction confined to a meningitis which is a part of a generalized process involving all the exposed pial surfaces of the brain and probably

does not reflect any changes within the bulb itself. There is no visible evidence of virus invasion in the bulb itself. The mitral cells are intact and show the picture seen in the control bulb (Pl. I, fig. 2) after long standing tract section.

The series of cases just presented represents a number of experiments in which the virus was administered in the simplest fashion—by mere instillation into the nose. Under these conditions virus usually reaches the central nervous system in sufficient concentration to cause poliomyelitis, but the quantity is not always sufficient to produce a fatal disease. If, at the time of inoculation, the nasal mucosa is slightly traumatized (i.e., rubbed gently with a pipe-stem cleaner)

TABLE II

*Unilateral olfactory tract sections—virus rubbed into nose**

EXPERIMENT	OPERATION	LESIONS OF POLIOMYELITIS	
612	Rt tract frozen 1 day	Rt bulb ++++	Lt neg, see Table III
609	Rt tract frozen 2 days	Rt bulb ++++	Lt see below
645	Rt tract cut 10 days	Rt bulb ++++	Lt see below
645	Lt tract cut 20 days	Lt bulb ++	Rt ++++
849†	Lt tract cut 25 days	Lt bulb 0	Rt ++
609	Lt tract cut 32 days	Lt bulb +	Rt ++++
A59	Rt tract cut 90 days	Rt bulb ++	Lt ++++

* See footnote Table I.

† Inoculated with the DuBois strain (Howe and Bodian, 1939).

the febrile reaction is more severe and the disease seems to progress more uniformly to complete prostration. It seems highly probable that under these conditions virus is brought into contact with more olfactory nerve fibers, since these fibers alone in the nose are susceptible to intranasal inoculation in this animal (50 cases, unpublished). Whether the effect is due to direct trauma from the pipe cleaner or more extensive efficient spread of the inoculum into the narrower portions of the olfactory area cannot be determined. At any rate, as Table II indicates, the sparing effect upon the mitral cells after olfactory tract section is less marked after this type of inoculation. It is significant, however, that although one finds a more extensive invasion of the operated bulbs than in the first series of cases, the same trend is apparent—there is a diminution in the severity of the

lesions after a two week post-operative interval. This may be seen whether the chronically isolated bulb is compared to the recently isolated one, or to the normal control bulb. It would thus appear that the ability of the mitral cells to resist destruction is to some degree dependent upon the amount of virus to which they may be exposed. This point will be considered again in another connection.

The amount of destruction produced by poliomyelitis virus in the mitral cells appears not only to be conditioned by the virus concentration which is built up in the olfactory bulb, but also is influenced by the degree to which the mitral cells show a reaction to the section

TABLE III

EXPERIMENT	OPERATION	LESIONS OF POLIOMYELITIS	
A. Unilateral olfactory tract sections close to brain—virus rubbed into nose*			
568	Lt tract cut 3 mos.	Lt bulb ++	Rt bulb ++++
994	Rt tract cut 3 mos.	Rt bulb ++++	Lt bulb ++++
A60	Rt tract cut 3 mos.	Rt bulb ++++	Lt bulb ++++
A61	Rt tract cut 3 mos.	Rt bulb +	Lt bulb +
B. Unilateral olfactory tract sections close to bulb—virus rubbed into nose*			
612	Lt tract cut 30 days	Lt bulb 0	Rt bulb ++++
A58	Rt tract cut 3 mos.	Rt bulb +	Lt bulb ++++

* See footnote Table I.

of their axones. This may be seen in Table III, A. When the olfactory tract is sectioned close to its exit from the olfactory bulb (*i.e.*, close to its cells of origin) the axone reaction in the mitral cells is more severe than after section of the tract at its midpoint or near its caudal termination. If the olfactory tract is sectioned very close to its caudal entrance into the brain, not only is the axone reaction less severe but some aberrant fibers may be missed and the animal may even contract poliomyelitis. This occurred in 994 and indicates that in this animal many cells were not affected at all by the tract section. This difference in the degree of axone reaction is reflected in the amount of resistance to virus destruction. Thus in the series of four cases in which the axone reaction was slight, there was little difference

between the operated and control sides even though a considerable time interval elapsed between operation and inoculation. In the two cases where the olfactory tract was sectioned close to the olfactory bulb and the reaction in the mitral cells was severe, invasion was very slight on the operated side though maximal in the control bulbs (Table III, B).

The experiments just reviewed seem to indicate quite clearly that the induction of an altered metabolic state in the mitral cells by section of their axones renders them virus resistant, but that such a resistance is relative and is conditioned by the concentration of virus and the degree of the change effected. However, one objection can be raised, namely that the operation of olfactory tract section has in some way produced a mechanical block to passage of virus from the olfactory mucosa to the bulb. As previously described (5) the operations were performed through the medial wall of the orbit so that there was no disturbance of the blood supply to the bulbs or of their relationships with surrounding structures. However, in order to eliminate even this remote possibility a series of four animals was prepared with a unilateral olfactory tract section of 6 weeks' duration. The dura covering the olfactory bulbs was then exposed by a frontal approach and each bulb was inoculated with 0.1 cc. of MV virus in piqûres (2 cases) or 0.05 cc. MV virus in 1 piqûre (2 cases). Treatment of the normal and operated bulbs was as nearly identical as possible. The animals were killed after they developed paralysis. In two cases which received one piqûre per bulb, the bulbs were sectioned serially *in situ* with the surrounding tissues of the nose. This ensured the same orientation of section in each bulb and facilitated more accurate comparison. Plate V shows such a pair of bulbs. The normal side (fig. 1) is maximally invaded with obliteration of its entire architecture while on the operated side (fig. 2) there is considerable sparing of mitral cells. The photographs were taken from corresponding portions of the bulbs in the same section and were selected to show the maximal contrast between the two sides. All the cases were similar, but in some there was more invasion of the isolated bulb than is shown here. Mitral cells, however, were 2 to 5 times as numerous by count on the operated side in every case. The chief difference between the normal and operated bulbs lay in the fact

that in the former, destruction was maximal throughout the bulb, while in the latter it was patchy as one would expect if the virus did not find conditions suitable for ready growth. The isolated bulbs which received two piqûres showed more general invasion than those receiving only one, which constitutes a further demonstration that resistance is relative and may be broken down by excessive concentration of virus.

Since the olfactory tract is a portion of the central nervous system no effective regeneration is possible, although abortive attempts are made. Four days after the axones are interrupted by freezing they are seen to have formed bulb-like expansions as the first stage of regenerative activity. Perhaps because the neuroglia of the tract blocks the growing tips of the fibers, they never succeed in crossing the gap presented by the frozen segment. It may be suspected that since the mitral cells are known to persist for many months, either regenerative efforts continue during this time, or their persistence is due to the presence of axone collaterals in the olfactory bulbs. No attempt has been made to determine the duration of the virus-refractory state since it may be assumed under these conditions to persist as long as the cells themselves. This portion of the problem has been more effectively studied in the cells of the spinal cord which give rise to peripheral nerve fibers in which all phases of regeneration may be more readily studied.

THE MOTOR CELLS OF THE SPINAL CORD

The second portion of this study considers the effects of axonal section upon another group of nerve cells highly susceptible to poliomyelitis virus, the motor neurones of the lumbar cord. It deals with 43 cases of nerve section. Thirty-three of these were subjected to cutting or freezing of one sciatic nerve in the thigh at a point proximal to any of its large branches. Eleven animals had sections of several lumbar nerve roots on one side. Six of these were extradural operations on the upper lumbar roots and five were sections of the lower sacral and caudal roots performed at the same time as sciatic section. Nine of the animals were subsequently inoculated, two being used as controls. This group was designed to contrast the effects of nerve sections at various distances from the cell body. Twelve animals

from the first series of simple sciatic sections received no virus but were killed at various intervals as controls. Thus, in the two series twenty-six animals were finally infected with poliomyelitis virus by either intranasal or intracerebral inoculation. Inoculations were so timed that complete paralysis was induced in the normal leg, and in the normally innervated muscles of the operated leg, 3 to 108 days after nerve section.

Following sciatic section the motor cells of the sciatic nucleus in the spinal cord show characteristic changes. Within three days, the Nissl bodies are slightly smaller than normal and the staining reaction to basic aniline dyes is paler (1+ chromatolysis, Plate VI, fig. 1). By the 6th day the size of the Nissl bodies is reduced by half in the majority of the cells, especially in the perinuclear zone, and there is a tendency for concentration of Nissl material at the periphery of the cell. The nucleus may also be slightly eccentric in the cell (2+ chromatolysis, Plate VI, fig. 2). Some cells show further reduction in the size of the Nissl bodies around the nucleus to dust-like particles, which may show almost no color whatever with such stains as gallocyanin, while at the periphery of the cell there is present a dense ring of deeply stained Nissl material. The nucleus is pale and eccentricly placed (3+ chromatolysis, Plate VI, fig. 3). This condition is found in the majority of the cells at ten days, after which there is a gradual reappearance of particulate Nissl substance and a reduction of the peripheral ring. The nucleus finally returns to the center of the cell. Only a few signs of chromatolysis can be found after functional recovery, but in the absence of complete regeneration it can persist as long as 90 days (1+). Section of motor roots or of mixed roots just distal to the spinal canal results in a more rapid and severe form of chromatolysis. After four days half of the cells show 2+ chromatolysis while changes are more extreme in the others, in which practically no stainable material remains in the perinuclear zone (3+ chromatolysis). The majority of the cells show this extreme form of reaction in 6 to 7 days.

The results of the sciatic nerve sections are summarized in Table IV. In the first four days there was no appreciable difference in the resistance of the cells of the operated and control sides. Plate VIII, figure 1 shows a typical case in which scarcely any anterior horn cells remained

on either side. In fact the process of neuronophagia seemed to be more advanced on the operated side, as though the susceptibility of

TABLE IV

Fate of anterior horn cells after sciatic nerve section

EXPERIMENT	DURATION OF SECTION	DEGREE OF SPARING		CHROMATOLYSIS
		Normal cells	Operated cells	
	days			
A25	3	No virus	No virus	+
A35	3	No virus	No virus	+
973	3	No virus	No virus	+
975	3	0	0	
983	3	0	0	
A172	4	No virus	No virus	+
A16	4	0	0	
804	6	No virus	No virus	++
939	6	0	0	
942	6	0	0	
A169	7	No virus	No virus	++
940	7	0	0	
941	7	0	0	
995	7	+	+	+++
978	8	++	++	+++
516	9	No virus	No virus	++
A190	10	No virus	No virus	+++
A34	12	+	+	+++
A12	13	0	+++	+++
A171	14	No virus	No virus	++
A33	16	0	+++	++
A57	17*	No virus	No virus	+
A55	17*	0	++++	++
998	17	+	++	+++
999	19	0	++++	+++
A170	21	No virus	No virus	++
A0	25	0	++++	++
A24	41	0	++++	++
A29	57	0	++++	++
A28	91	0	++++	+
A56	108*	0	++++	+
A54	108*	No virus	No virus	Trace only

* Freeze.

these cells had been enhanced by the section of their axones. Such a finding offers a ready explanation for the apparently facilitating influence of surgical operation or trauma upon the development of polio-

myelitis. From the sixth to the eighth days no consistent sparing was noted in the anterior horn of the operated side although the cells did not appear more susceptible than those of the normal side. At twelve to sixteen days a considerable number of cells was undestroyed on the side of sciatic section. This was all the more striking because of the complete destruction of the corresponding cells on the unoperated side. The spared cells all showed advanced chromatolysis—that is, their Nissl bodies were reduced to dust-like particles except for a ring around the periphery of the cell, and the nucleus was pale and eccentric. The chromatolytic cells were confined to the area of sciatic nerve cell distribution. All of the motor cells giving rise to somatic nerves other than the sciatic were destroyed and in addition to these many of the cells of the sciatic nucleus itself. The cells which were spared lay in that level of the cord where the majority of the cells were concerned with the formation of the sciatic nerve, while there was little sparing of the scattered cells in the rostral and caudal portions of the sciatic nucleus.

From the seventeenth to the ninety-first day, sparing became more extensive in the sciatic nucleus of the operated side. This was particularly striking in the four cases representing the 25th day (Plate VII, fig. 2), 41st day, 57th day (Plate VIII, figs. 1 and 2), and 91st day (Plate IX, figs. 1 and 2). Here, despite the fact that the cells of the normal sciatic nucleus were completely destroyed, the great majority on the operated side were spared. This was notable in view of the total destruction of other neighboring motor neurones on the operated side which were not involved in the sciatic section. This phase of almost maximal refractoriness coincided with the diminution of chromatolysis and the gradual reconstitution of the Nissl bodies (see Table II). In the ninety-one day sciatic section (A28, Plate IX, figs. 1 and 2), the Nissl bodies were only very slightly smaller than normal although the refractoriness of the cells was very great.

It may be noted in Table IV that cells surviving infection invariably show more advanced chromatolysis than those of uninoculated control animals of corresponding age. This constitutes some evidence that the refractory cells are visibly affected by the activity of the virus, either through its proximity to unprotected regions or by sublethal invasion of the resistant cells themselves. Chromatolysis is also one of the early signs of virus damage in unprotected cells, but here may

represent a reversible change which does not necessarily presage the death of the cell.

The animals with root sections (Table V) presented an interesting supplement to the sciatic sections. Within four days, 50 per cent of the surviving cells showed maximal chromatolysis, and within 7 days, the incidence was 60 to 90 per cent. Control cords without virus showed maximal chromatolysis within one week instead of at the end of the second week as in the case of sciatic nerve sections. As might be expected, sparing of cells was greater in these cords than in those

TABLE V

Fate of anterior horn cells after root section

EXPERIMENT	DURATION OF SECTION	DEGREE OF SPARING		CHROMATOLYSIS
		Normal cells	Operated cells	
	days			
A1	4	0	+	+++
A2	4	0	+	+++
A3	4	0	+	+++
A4	4	0	0	
804	6	No virus	No virus	
939	6	0	++	+++
942	6	0	++	+++
A143	7	No virus	No virus	+++
940	7	0	+	+++
941	7	0	+	+++
A185	7	0	+	+++

of the same time interval showing less chromatolysis. It was also greater after seven days of root section than after four. The last four animals of the series also had sciatic sections as well as root sections below the sciatic distribution. The cells of the lower sacral and caudal cords showed much more extensive sparing than was seen in the sciatic nucleus despite the fact that there was no difference between these cell groups on the control side. The root sections constitute a further demonstration that the sparing effect is proportional to the degree of reaction induced in the nerve cell by axonal section.

It happened that during the early phases of this study, in all the long standing sciatic sections there was no indication that muscle groups had been reinnervated. No attempt had been made to reap-

proximate the cut ends of the nerve so that at the time of inoculation no effective regeneration had taken place. It therefore seemed important to determine whether the resistance of the motor cells outlasted their complete functional recovery. Accordingly two animals were prepared by freezing the sciatic nerve of one side at the level ordinarily used for section. It had been previously shown in animal A 55 that freezing the nerve trunk with dry ice was qualitatively the equivalent of nerve section for the induction of refractoriness in the sciatic nucleus, but that probably owing to the greater speed of nerve regeneration the cycle was of shorter duration so that very substantial protection was attained as early as 17 days. After the sciatic nerve was frozen just below the sciatic notch, 100 days elapsed before the flexors and extensors of the feet were again innervated and sensation had returned to the toes. At this time one animal (A56) was inoculated intracerebrally and became completely paralysed in the legs 108 days after the nerve freeze. The control was killed on the same day. Sections of the lumbar cord of A56 showed no sparing whatever on the operated side (Plate X, figs. 1 and 2). In the control animal the cells of the sciatic nucleus could scarcely be distinguished from those of the normal side with regard to the size and staining reaction of their Nissl bodies (Plate XI, figs. 1 and 2).

As in the case of the olfactory bulb it was shown that the resistance of the anterior horn cell is conditioned by the quantity of virus available and the degree of reaction induced in the nerve cell. For example, if the freshly cut central end of the sciatic nerve be immersed in virus emulsion, each fiber is brought into contact with the infective agent and severe poliomyelitis results after a minimal incubation period (8). Histological examination of the lumbar cord in such a case reveals complete destruction of all the motor cells of both sides. When, however, a sciatic nerve is cut on three successive occasions with a two week interval between each, and is finally recut and soaked in virus, the resistance of the cells of the sciatic nucleus is so high that in some cases (6 out of 9) the animal does not contract poliomyelitis (9). The three cases in which the animals became paralysed showed extensive sparing of the motor cells in the sciatic nucleus with destruction of all others in the cord (Plate XII, fig. 1). Not only was a large number of cells spared but an unusually uniform degree of chroma-

tolysis was apparent. After a single nerve section it is customary to find cells in all stages of regenerative activity—some showing early, others advanced, and still others late chromatolysis. After three successive nerve sections all the cells are found in a late phase of chromatolysis at which time they are maximally refractory to virus. Plate XII, figure 2, shows the sciatic nucleus of an uninoculated animal after three successive nerve sections and demonstrates this very clearly, with two normal cells of some group other than that of the sciatic nucleus for comparison.

The interpretation of the period of incomplete sparing during the second and third weeks (see Table IV) now becomes much simpler. Section of the sciatic nerve in the thigh does not interrupt all axones at the same point relative to the cell body. Hence, it follows that the axone reaction will be most severe in the cells which possess the longest axones, and least severe in those whose axones were interrupted relatively closer to their terminations. Thus one finds cells exhibiting almost all degrees of chromatolysis after a simple nerve section. Under these conditions considerable numbers of cells have reacted sufficiently to become resistant to virus reaching the nucleus via the pathways of the spinal cord, but the lightly chromatolysed cells apparently cannot withstand the higher virus concentrations resulting from immersion of all the axones in virus. Repeated nerve section apparently increases resistance by enhancing the axone reaction in cells which would be only lightly affected by one nerve section. The strengthening of the weakest units thus exercises a further effect upon the resistance of the group as a whole.

DISCUSSION AND SUMMARY

It has thus been shown that axonal section is sufficient to render two different groups of highly susceptible nerve cells refractory to destruction by poliomyelitis virus. Both in the olfactory bulbs and in the anterior horns a period of a week elapses before much change can be noted in the reaction of the tissue to virus. Following this there ensues a period of increasing refractoriness which reaches a maximum in about 3 weeks and may apparently persist as long as effective regeneration does not occur. Since in the olfactory bulb regeneration is not possible and the mitral cells may persist for at least 9 months, the

state of refractoriness may be assumed to be of considerable longevity; nevertheless it is abolished if, as in the anterior horn cells, regeneration is finally effected. Furthermore, the ability of the regenerating cell to resist destruction is conditioned to some extent by the degree of its regenerative activity and the amount of virus to which it is exposed.

As far as we are aware no strictly comparable phenomenon has been previously described. The resistance shown to electrolysis and to certain chemicals by regenerating cells in the nasal mucosa, kidney, and liver as described by various observers (10–12), depends upon the replacement of the original epithelial cells by new individuals of a less differentiated type. Obviously, this is not the case in our experiments.

In the beginning of this investigation it was very tempting to ascribe this refractoriness of the nerve cell to a phase of the metabolic change ensuing during regeneration of its axone, which is mirrored in the well-known histologically demonstrable reaction of chromatolysis. Later it became apparent that the degree of histological change did not truly parallel the metabolic alteration essential to the refractory state of the cell, since this latter state was not fully developed when chromatolysis had reached its maximum and attained its peak when chromatolysis was on the wane. One important difference exists between the mitral cells and the anterior horn cells. In the former, regenerative efforts take place, but are blocked presumably by the inability of the fibers to cross the mechanical gap of the tract section. The cells finally reach a state of equilibrium in which the cell body is smaller and the amount of Nissl substance less than normal. This is a state in which the cells are highly refractory to virus and it may be presumed to be one of considerable duration, since it is known that the mitral cells persist in this form for at least nine months. On the other hand the peripheral nerves of the monkey regenerate readily, so that in the anterior horn cells the virus refractory state is soon passed. If however the nerve is sectioned repeatedly, the cells are subjected to conditions more nearly comparable to those found in the olfactory bulb and the refractoriness to virus rises markedly. It seems very likely that in both of these cases the peak of regenerative activity has been passed with the peak of chromatolysis and that the period of refractoriness to virus in both groups of cells represents a new

equilibrium in the nerve cell at which its metabolism is readjusted at a new level. It is important also to realize that the change in the form of the stainable material in the cytoplasm of the nerve cell may have no connection whatever with those changes in metabolism which render the cell refractory to virus.

At the present time it is unknown whether the cells are invaded by virus but not destroyed, or whether they no longer offer a foot-hold for it. This question first must be settled in order to give direction even to speculation on the nature of the changes effected in the cells. As working hypotheses, one can consider alterations in oxidation-reduction potentials or in specific enzyme systems, decreases in membrane permeability or in particular cell proteins which may be essential for virus growth. The investigation of such factors may lead to some fundamental knowledge of the virus-host relationship.

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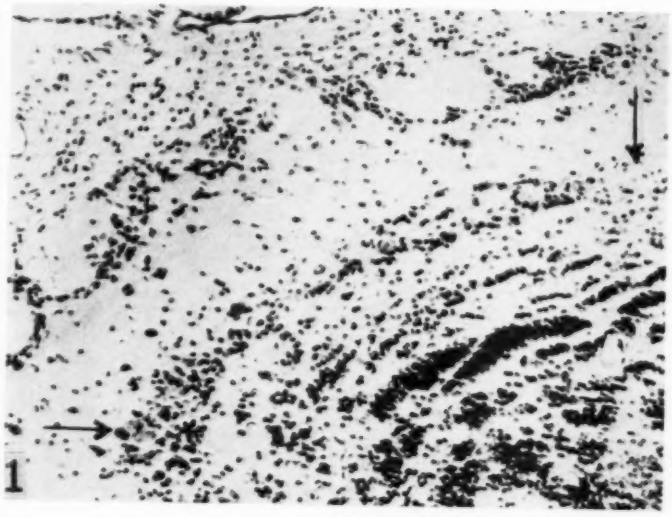
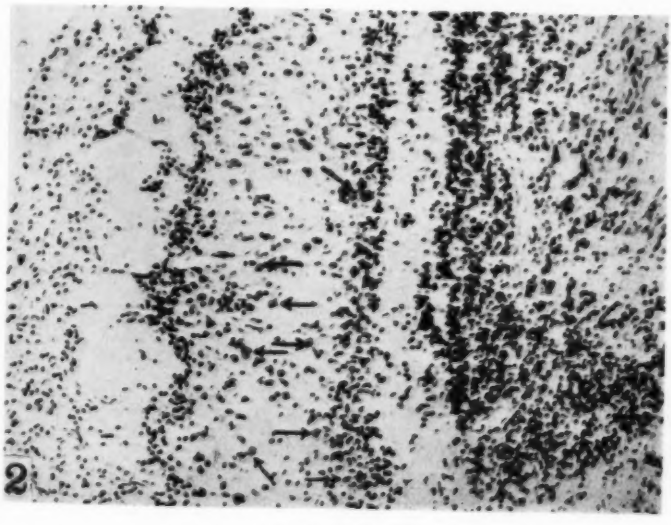
PLATE I

FIG. 1. Normal olfactory bulb of monkey 732a. Note the glomerular layer (in the upper left third of the picture), the relatively acellular molecular layer which contains a few mitral cells, the mitral cell layer, and the central area of granule cells. The mitral layer is indicated by arrows. × 130.

FIG. 2. Operated olfactory bulb of animal 732a seven months after olfactory tract section. Note the persistance of mitral cells at reduced size in the molecular and mitral layers. × 130.



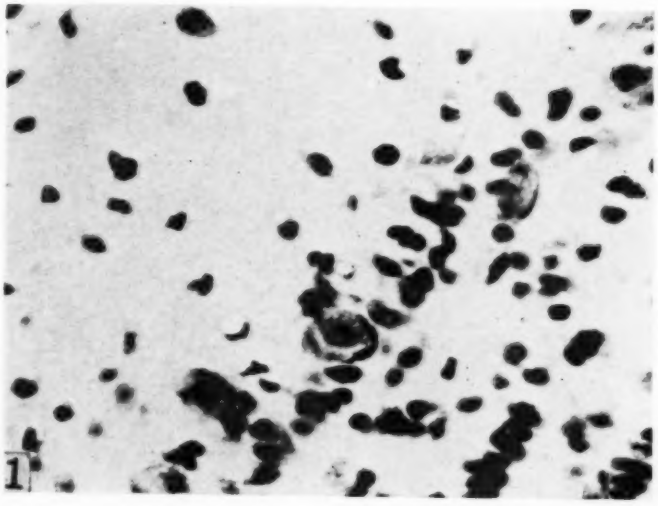


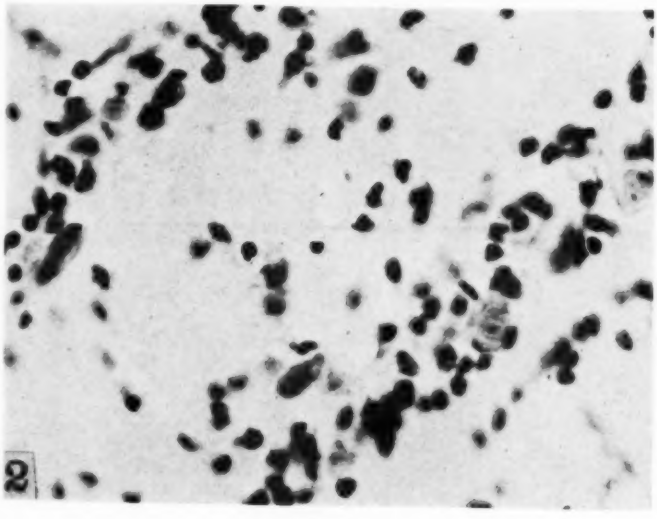
FIGS. 1–2

PLATE II

FIG. 1. Mitral cells from the normal olfactory bulb of 732a. Note the quantity of Nissl material in the cytoplasm of the cells. × 540.

FIG. 2. Mitral cells from the operated olfactory bulb of 732a seven months after olfactory tract section. Note the reduction in the size of the cells and in the amount of Nissl substance in their cytoplasm. × 540.



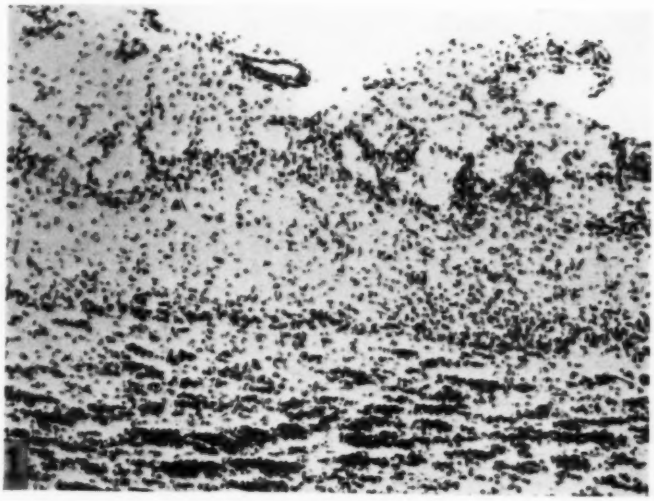


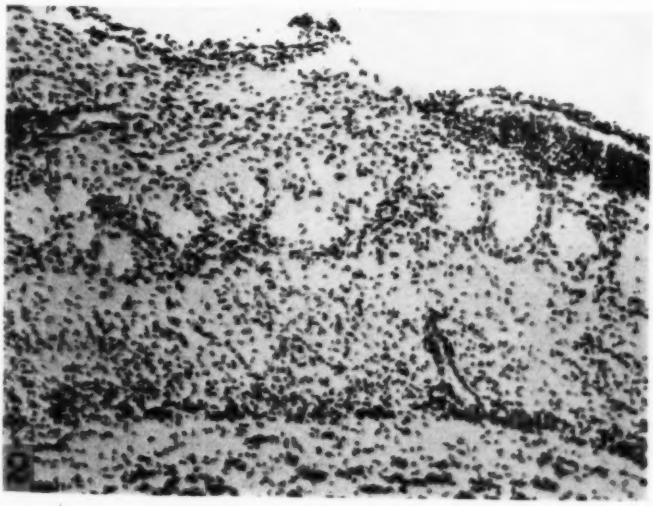
FIGS. 1–2

PLATE III

FIG. 1. Unoperated olfactory bulb (909) showing severe reaction following intranasal inoculation of Rockefeller MV virus. Note the obliteration of the normal layering by cellular infiltration, the perivascular cuffing, and the surface meningitis. These reactions are in large measure secondary to the destruction of the mitral cells which in this case has been almost complete. × 160.

FIG. 2. Operated olfactory bulb from animal 909 in which the olfactory tract had been cut 2 days prior to inoculation. Invasion of this bulb is as extensive and severe as in the normal bulb. × 160.



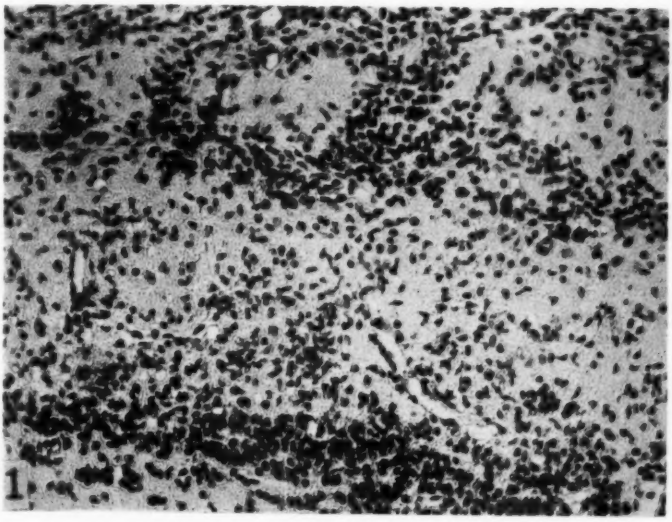


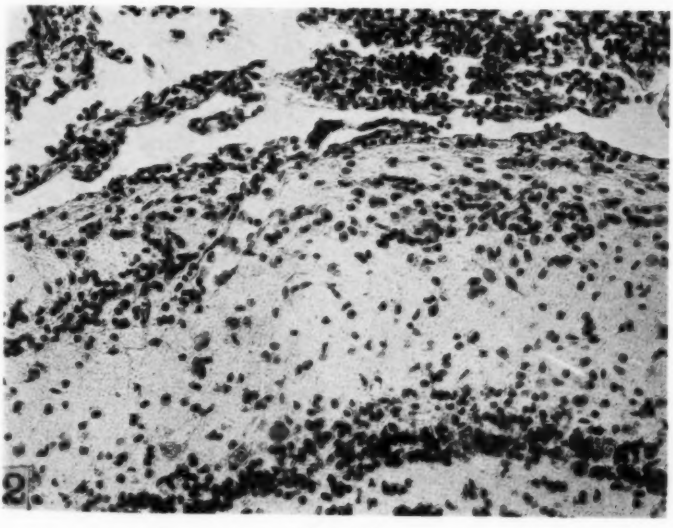
FIGS. 1–2

PLATE IV

FIG. 1. Unoperated olfactory bulb from animal 898 showing maximal invasion following intranasal inoculation. × 180.

FIG. 2. Operated olfactory bulb from animal 898. Olfactory tract section 15 days prior to intranasal inoculation. The only reaction is a surface meningitis which is common to other pial surfaces. Note normal mitral cells and absence of cellular infiltrations. × 180.



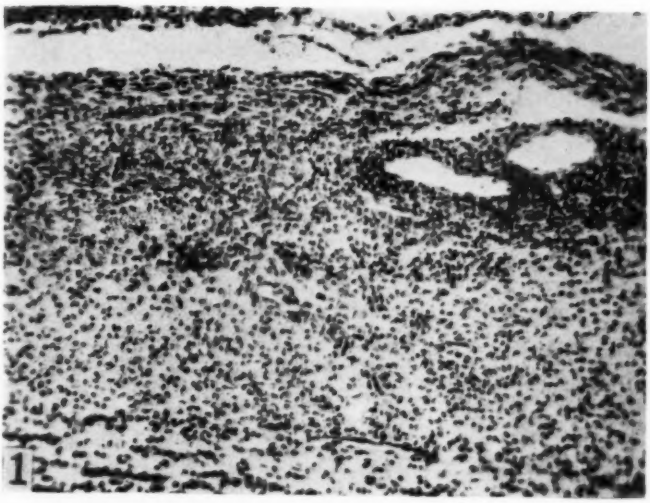


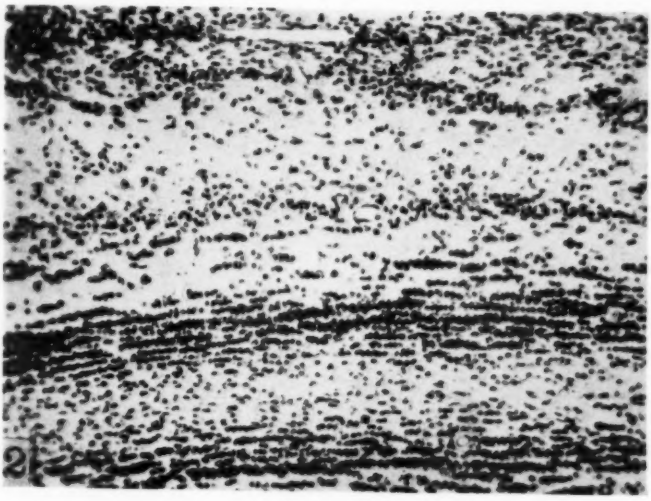
FIGS. 1–2

PLATE V

FIG. 1. Unoperated olfactory bulb from animal 919, inoculated by direct stab. Note complete loss of mitral cells and obliteration of normal architecture of the bulb by the poliomyelitic process. × 120.

FIG. 2. Operated olfactory bulb from animal 919 inoculated by direct stab 6 weeks after olfactory tract section. Note normal area in this bulb as contrasted with the unoperated side in which invasion was maximal throughout the entire bulb. × 120.





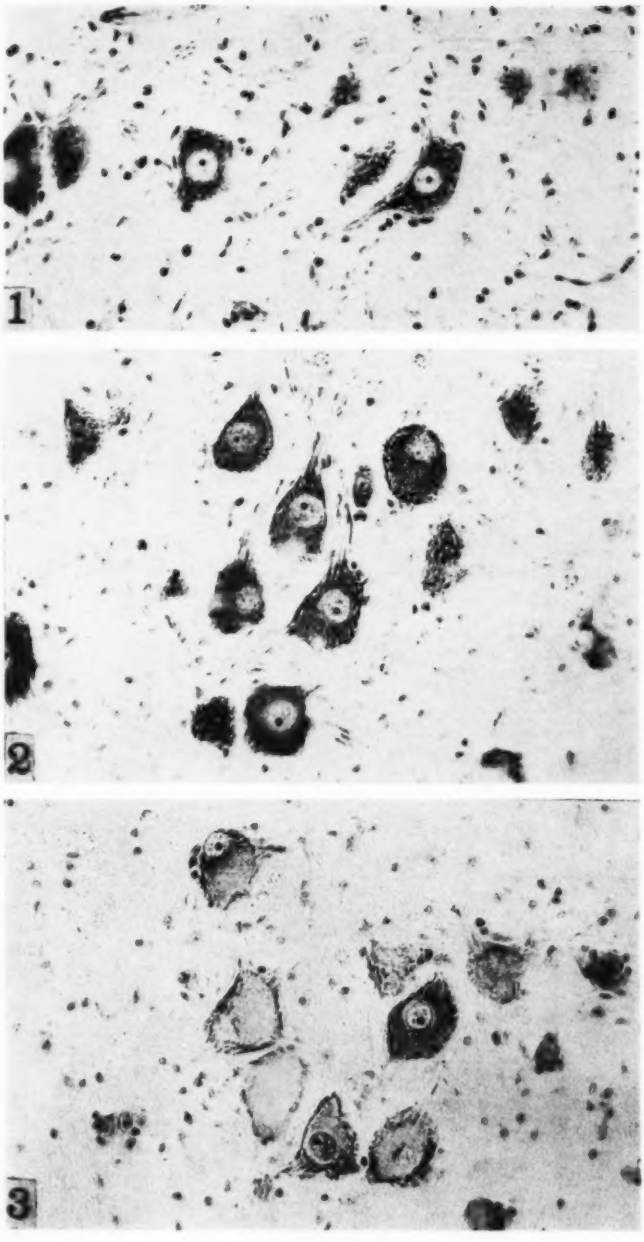
FIGS. 1–2

PLATE VI

FIG. 1. Early chromatolysis (+) in a motor cell of the lumbar cord 3 days after section of the sciatic nerve (A35). A normal cell is included for comparison. Note the reduction in the size of the Nissl bodies. × 210.

FIG. 2. Moderate chromatolysis (++) in motor cells of the lumbar cord 6 days after section of the sciatic nerve (804). Note the further reduction in the size of the Nissl bodies and the slight eccentricity of the nucleus. It is also clear that some cells show more advanced chromatolysis than others. × 210.

FIG. 3. Severe chromatolysis (+++) in the motor cells of the lumbar cord 7 days after section of the anterior division of L_4 (A143). There is now striking rarifaction of the Nissl substance which may be still present only at the cell periphery, and marked nuclear pallor and eccentricity. Chromatolysis of this degree is not seen until the 10th day following sciatic section. × 210.

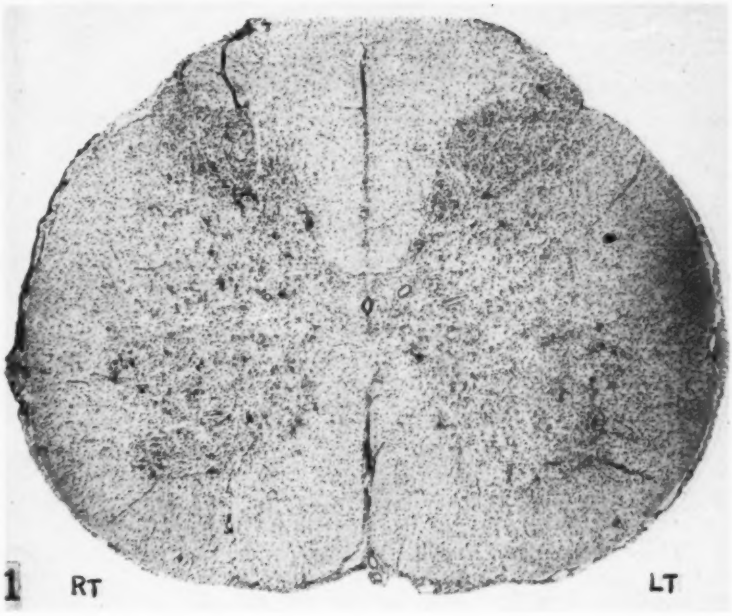


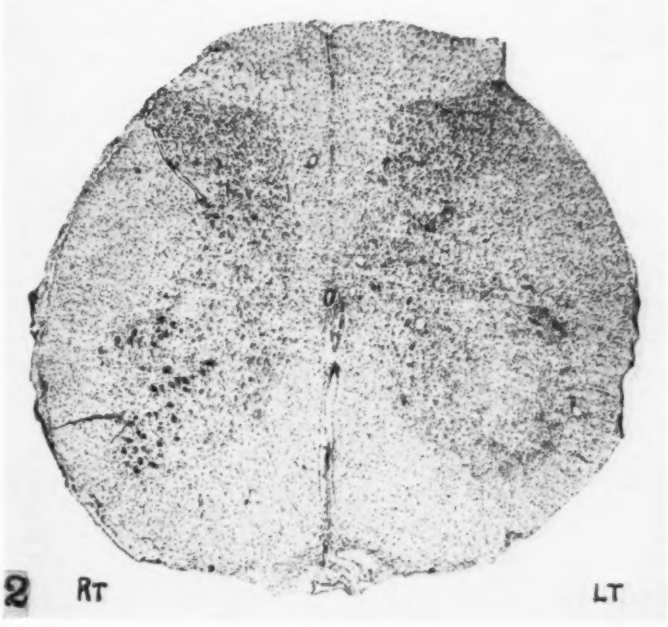
FIGS. 1–3

PLATE VII

FIG. 1. Complete destruction of the motor cells of the anterior horns in the lumbar region (975). The right sciatic nerve had been sectioned 4 days prior to the appearance of complete paralysis in the unoperated leg. × 17.

FIG. 2. Sparing of anterior horn cells in the right sciatic nucleus (AO). The right sciatic nerve had been sectioned 25 days before virus reached the lumbar cord in destructive concentrations. Note the complete destruction of cells on the control side. × 20.



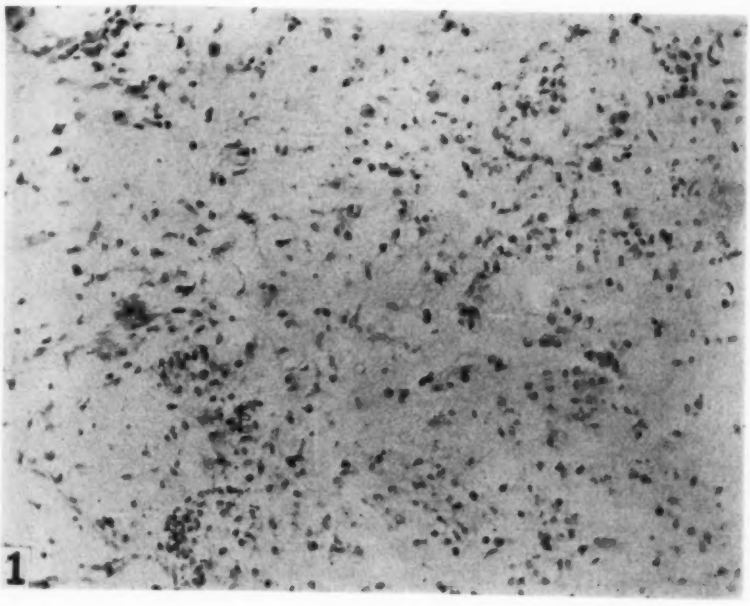


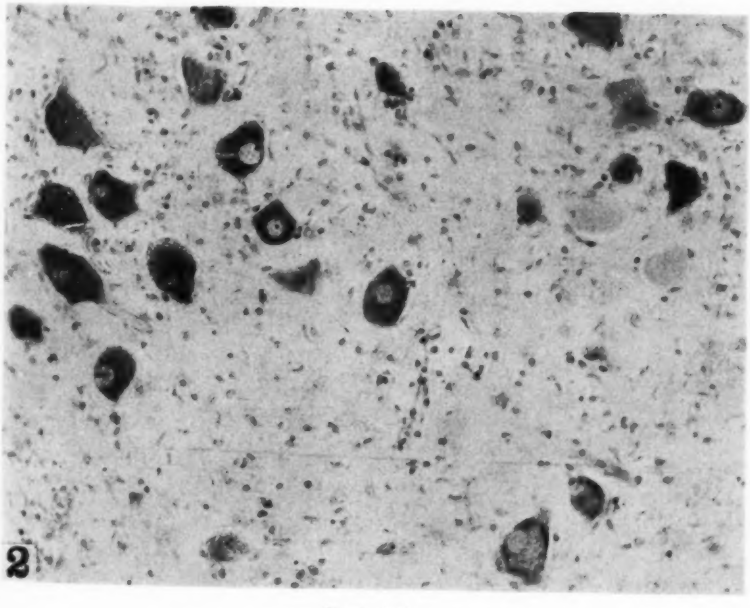
FIGS. 1-2

PLATE VIII

FIG. 1. Control anterior horn of A29 (left side) showing complete destruction of motor cells. × 175.

FIG. 2. Sparing of motor cells in the right sciatic nucleus of A29. The right sciatic nerve had been sectioned 57 days previous to paralysis. Note the regenerating Nissl bodies in the spared cells, which are showing ++ chromatolysis. × 175.



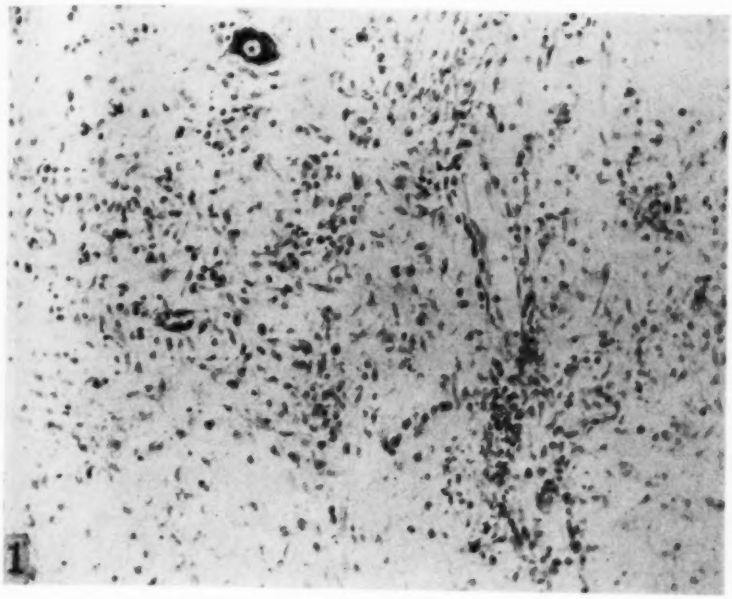


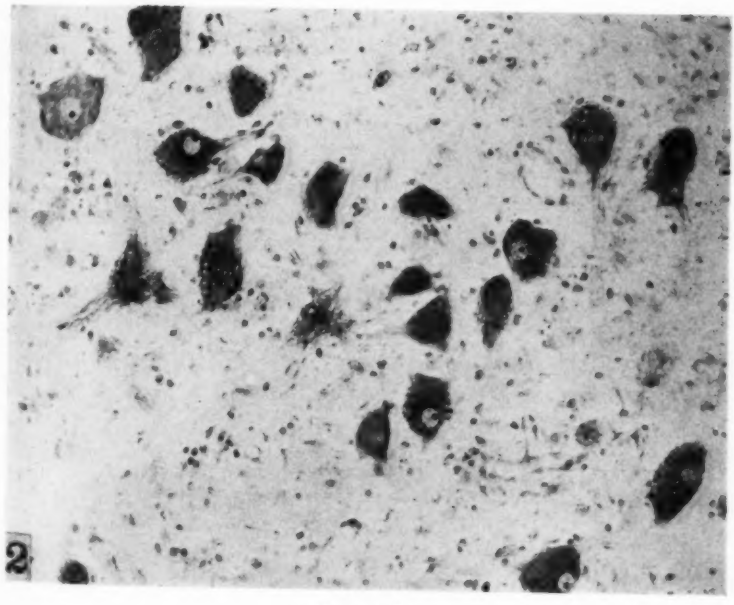
FIGS. 1–2

PLATE IX

FIG. 1. Control anterior horn of A28 (left side), showing complete destruction of motor cells. × 175.

FIG. 2. Sparing of motor cells in the right sciatic nucleus of A28. The right sciatic nerve had been sectioned 91 days previous to paralysis. Note that there is even more regeneration of Nissl bodies, reducing the amount of chromatolysis to a + level. × 175.



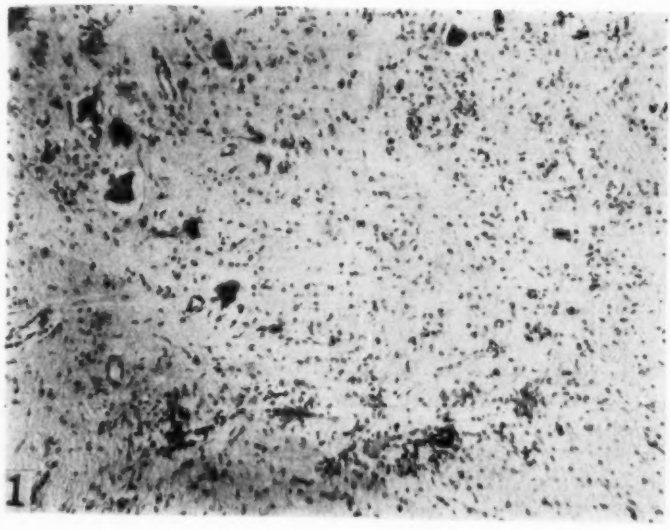


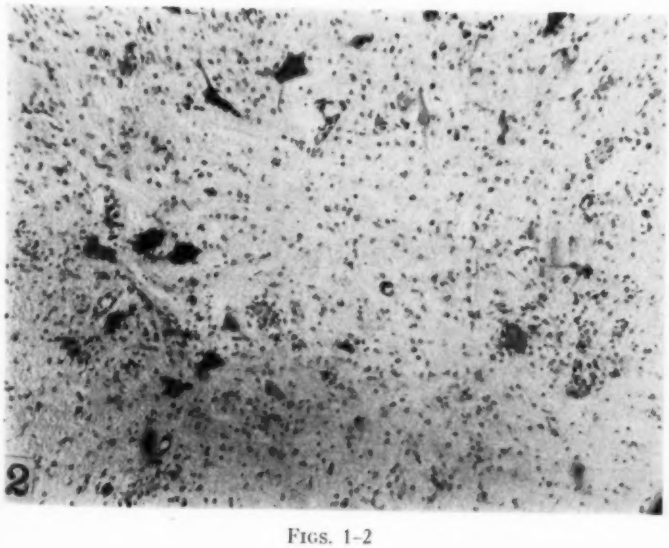
FIGS. 1–2

PLATE X

FIG. 1. Control anterior horn of A56 (left side) showing complete destruction of motor cells. × 90.

FIG. 2. Destruction of the right sciatic nucleus of A56. The right sicatic nerve had been frozen 108 days previously and there had been functional recovery before inoculation. × 90.



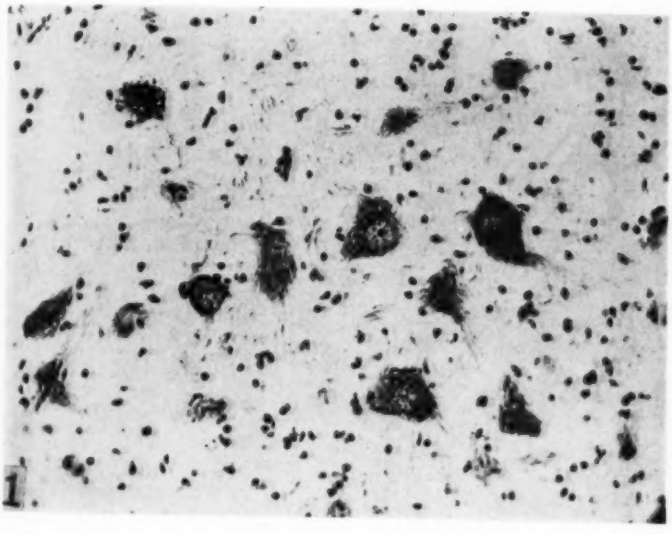


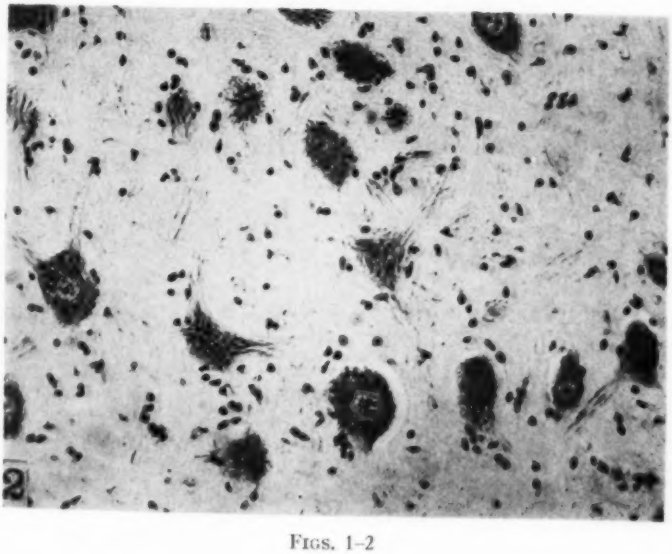
FIGS. 1-2

PLATE XI

FIG. 1. The right sciatic nucleus of A54 (control for A56). The right sciatic nerve had been frozen 108 days previously. Considerable functional recovery in the muscles of the foot had taken place before the animal was killed without inoculation. Note the normal appearance of the Nissl bodies. A few cells still showed slight chromatolysis despite the fact that in the figure the cells of the operated side cannot be distinguished from the normal side. × 90.

FIG. 2. Normal sciatic nucleus of A54 (left side). These cells cannot be distinguished from those shown on the regenerated side. × 90.



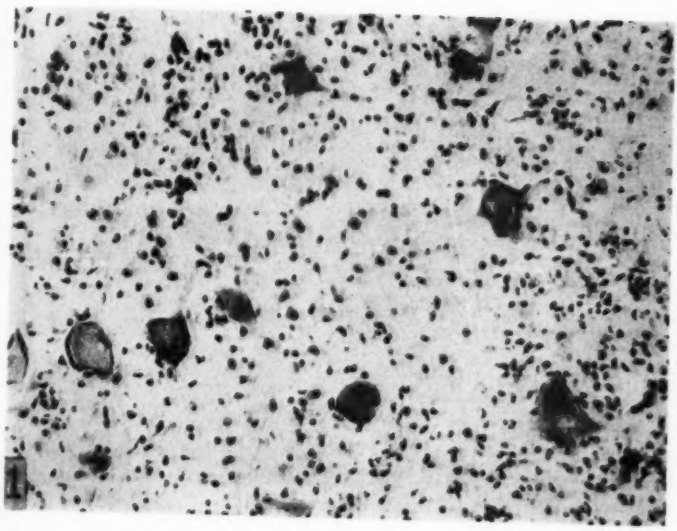


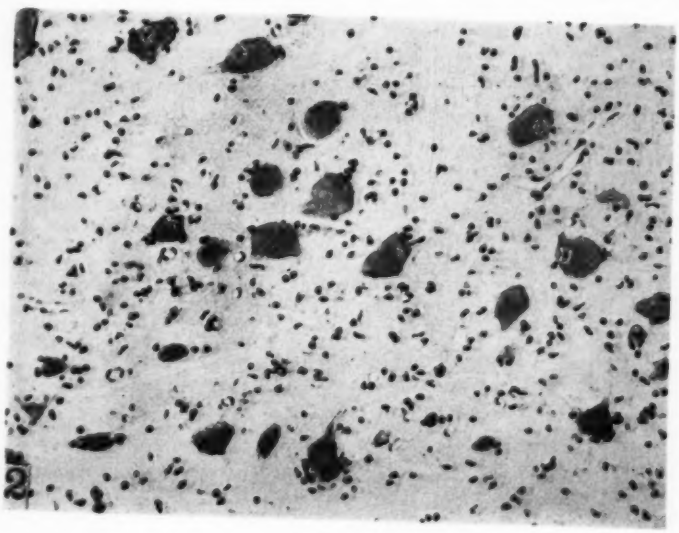
FIGS. 1–2

PLATE XII

FIG. 1. Cells of the sciatic nucleus following four successive sections of the sciatic nerve. The animal contracted poliomyelitis following final immersion of the proximal stump of the nerve in virus, but there was nevertheless extensive sparing of cells in the sciatic nucleus. × 160.

FIG. 2. Cells of the sciatic nucleus following three successive sections of the sciatic nerve. No virus. Note as in the above the uniformity of chromatolysis. Two normal cells are included for comparison. × 160.





FIGS. 1-2

UNIV. OF MICH.

THE PATHOLOGY OF EARLY ARRESTED AND NON-PARALYTIC POLIOMYELITIS[1]

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The existence of non-paralytic cases of poliomyelitis in man cannot be questioned (see survey of International Committee, '32, the review of Van Rooyen and Rhodes, '40, and Sabin and Ward, '41), although naturally the diagnosis may be uncertain in many cases. In the experimental animal it is possible to confirm the diagnosis of non-paralytic poliomyelitis histologically, but it is only recently that the importance of examining the nervous tissues of monkeys which fail to show paralysis after inoculation has been fully appreciated. The differentiation between an "abortive" type, with systemic symptoms but no involvement of the nervous system, and a "non-paralytic" type, with nervous system involvement but no paralysis, has not been critically established, since, in the absence of confirmation by means of histopathological material or by virus assay of nervous tissues, the former type can be only hypothetical. In the experimental animal one sometimes observes a febrile response following inoculation, with no subsequent paralysis and no demonstrable lesions in the nervous system on routine histopathological examination. It is obviously difficult to assign the designation of "abortive" or "systemic" poliomyelitis to such a case in a critical way, just as it has not as yet been possible to confirm the existence of a purely systemic disease in man. It would be necessary to show in either case that the occurrence of systemic symptoms was followed by the development of newly formed, specific antibodies, but that virus, or lesions resulting from the presence of the virus, was not present at any time in the nervous system.

The criteria for establishing the diagnosis of "non-paralytic" poliomyelitis in the experimental animal are easier to fulfill, since the

[1]Supported by a grant from the Commonwealth Fund.

presence of virus or lesions in the nervous system in an animal which had failed to become paralyzed is sufficient to establish the existence of this form of the disease. Nevertheless, before the work of Sabin and Ward ('41), carried out simultaneously with our experiments, there were few, if any, histopathological data available which could throw light on the following questions, all of which are of primary importance in any consideration of the mechanism of arrest of the pathological process in paralytic as well as in non-paralytic cases:

I. Can the virus of poliomyelitis enter the central nervous system and spread within it without production of demonstrable lesions or of clinical symptoms referable to the nervous system?

II. What is the minimal amount of pathological change which may be found in non-paralytic cases, and can the pathological process be arrested before the typical distribution of lesions in the brain, characteristic of paralytic cases inoculated by the same portal, is fully developed? It is known, of course, that the characteristic distribution of lesions can be prevented from fully developing by killing the animal in the pre-paralytic stage, but a description of cases in which the spread of lesions is spontaneously arrested in a very early stage has not yet come to our attention.

III. How far can the pathological process extend in the brain and spinal cord without being severe enough to produce clinically evident paralysis? Obviously, in mild cases the absence of demonstrable paralysis is no indication of the absence of severe though scattered destruction of anterior horn cells, but in the latter case, how extensive are the cerebral pathological changes?

I. CAN VIRUS SPREAD IN THE NERVOUS SYSTEM WITHOUT PRODUCING LESIONS?

The question of the possible existence of virus in the central nervous system without production of demonstrable pathological change, although of considerable importance, is in a certain sense largely theoretical, since it is not possible as yet to obtain virus from a bit of tissue without destroying the availability of that particular bit of tissue for histological study. Nevertheless, indirect evidence has been offered (Brodie, '34), and frequently quoted, that poliomyelitis virus can exist in the spinal cord in the absence of typical lesions in

other regions of the cord or brainstem. If accepted at face value, the single case presented by Brodie would seem to contradict an observation repeatedly reported by various observers (Fairbrother and Hurst, '30; Pette, Demme, and Környey, '32; and others) as well as by Brodie himself ('33). This is the finding that the infectivity of nervous tissues is proportional to the amount of actual neuronal damage, as measured especially by neuronophagia. However, a more serious objection to the full acceptance of the implications of Brodie's case, namely that the virus can exist in the central nervous system in infective doses without producing pathological change, is the fact that one can find severe lesions, including considerable neuronophagia, in one segment of the spinal cord, in the absence of any observable histopathological change in a neighboring segment as close as 1 or 2 millimeters (see also Sabin and Ward, '41). This we have observed in non-paralytic cases, described in more detail below, and it undoubtedly occurs in cases of mild degree of paralysis. It is therefore apparent that the diagnosis of the presence of virus in the nervous system without the presence of pathological change, cannot be accepted without critical proof. It is at least necessary to establish the absence of lesions in serial sections of the entire cerebrospinal axis, and this has not as yet been done. There is also no evidence that the virus, when present in the central nervous system in sufficient quantity to spread therein, does not produce pathological change, although such changes are rarely seen in the fiber tracts along which spread occurs, but principally in the synaptic stations. In view of the known rapid decrease of infectivity of nervous tissues of poliomyelitis cases, it is more than likely that if a quantity of virus reaching a susceptible center in the central nervous system is insufficient to set up the local pathological response which precedes the spread of lesions in the central nervous system, it will be destroyed locally before spreading to any appreciable extent. Stated in another way, it is improbable that virus entering the central nervous system in smaller quantity than the minimal infective or pathogenetic dose for susceptible cells near the portal of entry, is capable of multiplying or spreading to any considerable degree before being sterilized locally. The minimal infective dose varies, of course, depending not only on the susceptibilities of different host individuals, but also on the varying susceptibil-

ities of the particular nervous centers which are first invaded by the virus.

II. THE MINIMAL DEGREE OF SPREAD OF PATHOLOGICAL CHANGE IN ARRESTED CASES

When virus reaches the central nervous system it may be assumed that one of several sequels is possible. If the dose of virus is subinfective for any nerve cell at or near the site of entry, the virus may be sterilized there, with no demonstrable lesions being produced. If the dose of virus is infective for neurons at or near the place of entrance to the nervous system one of two results may be expected. Either a local pathological response to the invasion and injury of nerve cells at the site of entry may occur, followed by sterilization of the virus before it can multiply sufficiently to spread to other susceptible centers, or else the multiplication and spread of the virus leads to invasion of all susceptible centers, resulting in the definitive distribution of lesions characteristic of the typical paralytic case (Bodian and Howe, '40). Between these two alternatives, all things being equal, should lie all degrees of spread of virus and of pathological reaction, short of paralysis. The more advanced cases of arrested, nonparalytic poliomyelitis will be considered below. It is necessary to inquire here whether the minimal degree of pathological change resulting from entrance of an infective dose into the nervous system can be a local response at the site of entry, with local arrest of the process and failure of development of the characteristic pattern of distribution of the encephalitic lesions. If this does occur it means that the pathological diagnosis of poliomyelitis in early arrested cases depends on a thorough examination of the region of the nervous system to which the virus first gains access.

There have come to our attention cases of poliomyelitis in two chimpanzees and in three rhesus monkeys which contribute to this problem. The chimpanzees have been described in detail elsewhere (Howe and Bodian, '41) and will be discussed here only briefly.

The first chimpanzee, a four year old, vigorous male (A 48) was inoculated on three successive days by the intra-oral administration of 2 cc. of a human stool suspension known to contain potent poliomyelitis virus. One month later, a febrile reaction (106°F.—normal 101°F.) occurred, but the rectal temperature

returned to normal the following day. At this time, cerebrospinal fluid obtained by lumbar puncture clotted immediately, but a pleocytosis was established by staining the clot with methyl green. The next day a mild left facial paralysis, with eyelid ptosis, was noted. This symptom improved in the next few days, during which time the animal appeared to be essentially normal in all other respects. He was killed four days after the onset of facial paralysis. This case is included in the series of non-paralytic cases to be described because of the minimal degree of paralysis which occurred and because of the remarkable nature of the histopathological findings. Serial sections of the brain, cranial ganglia, and selected blocks of spinal cord at cervical, thoracic, and lumbar levels revealed that this case was essentially arrested, so far as pathological findings are concerned, at or near the region of entrance of virus into the brain (see Howe and Bodian, '41, Plate IV). Characteristic lesions, including perivascular cuffing, leucocytic infiltration, and neuronophagia were found exclusively in the following centers: Gasserian ganglia, especially the left one, the left sensory nuclei of the trigeminal nerve, the left facial nucleus and the cervical cord; single cuffed vessels were noted in the left tractus solitarius, the right motor nuclei of the trigeminal, facial, and hypoglossal nerves, and the right inferior olivary nucleus.

These findings demonstrate, first, that the route of entry of the virus into the brain was apparently via the trigeminal roots and perhaps also along the vagal roots, and second, that *the pathological process was arrested before spread of virus had occurred to involve any susceptible brain or spinal cord centers outside of the medulla oblongata and cervical cord.* The fact that a minimal degree of facial paralysis was present is obviously incidental to the fact that the pathological process was arrested after minimal involvement of brain stem centers in the region of virus invasion of the brain. This occurred despite the fact that sufficient neuronal damage occurred in the left facial nucleus to permit multiplication of the virus which gained entry. It was perhaps accidental that this neuronal damage was just sufficient to produce clinically demonstrable paralysis. Especially noteworthy is the fact that in this mild paralytic case, and also in some of the non-paralytic cases to be described, *the full development of the characteristic distribution of encephalitic lesions, such as is found in the overwhelming majority of cases in monkeys, chimpanzees, and man* (Bodian and Howe, '40; Howe and Bodian, '41), *failed to occur.* It is obvious that autopsies of human cases with arrest of the pathological process at such an early stage would be extremely rare, and to the best of our knowledge such cases have not been described.

The second chimpanzee, an adult tuberculous male, with severe cage paralysis of all extremities (A 175), was inoculated intranasally with the same material used in the previous case, 1 cc. being instilled into each nostril. This was done on October 18, 29, 30, and 31, 1940. During the following days his temperature was irregular, and he apparently had a tuberculous pneumonia. He died on November 5, without having shown any signs of poliomyelitis. Serial sections of the brain, cranial ganglia, and cervical and lumbar cord, revealed, however, numerous lesions characteristic of poliomyelitis in the olfactory bulbs and secondary olfactory centers, and in no other parts of the nervous system examined (see Howe and Bodian, '41, Plate II). Spinal cord sections showed some nerve cell atrophy and loss, characteristic of cage paralysis, but no lesions of poliomyelitis. The lungs showed far advanced tuberculosis, with bilateral tuberculous pneumonia and fibrous pleuritis. There was extensive caseous tuberculosis involving all of the thoraco-abdominal lymph nodes and most of the abdominal viscera.

This case is of considerable interest because, in addition to its similarity to the previous case, except in regard to the portal of entry of the virus, it represents the arrest of the pathological process of poliomyelitis in an extremely early stage, and localized to the region of the brain at and near the site of ingress, in an animal which received an inoculation that in a healthy animal (A 83, reported elsewhere; Howe and Bodian, '41) was sufficient to produce a severe polioencephalomyelitis. It must be seriously considered whether the arrest in this case was due to the pre-existing disease, either the tuberculosis or vitamin deficiency, or both.

The foregoing cases are sufficient to illustrate the fact that the disease process in poliomyelitis may be restricted to the region of virus entry. In addition to these cases, however, may be mentioned three rhesus monkeys which failed to show any paralysis after inoculation. These animals were inoculated intranasally with stool material obtained from a poliomyelitic chimpanzee (A 106—Howe and Bodian, '41). After a febrile response they failed to show paralysis and were sacrificed from one to three weeks after the temperature had returned to normal levels. All showed definite and typical lesions of poliomyelitis in sections of the olfactory bulbs, secondary olfactory centers, and hypothalamus, but in no other parts of the brain (A118, A146, A169). Serial sections of 6 mm. segments of the spinal cord at five or six levels contained no lesions. In short, the virus of poliomyelitis had produced a minimal degree of encephalitis,

localized to the region of ingress of virus into the brain, without involving the spinal cord.

III. MAXIMAL EXTENT OF PATHOLOGICAL PROCESS IN NON-PARALYTIC CASES

The problem of how far the pathological process in the central nervous system can extend before the disease is arrested in non-paralytic cases must be approached with the understanding that the clinical diagnosis of a non-paralytic case may rest on the failure to detect minimal degrees of muscle weakness. Furthermore, it is theoretically possible that anterior horn cells may be completely destroyed in a short segment of cord without resultant noticeable paralysis, because of the fact that a functional group or a single large muscle may be innervated from several segments, or from a considerable length of cord, and thus may retain apparently normal function when actually part of its innervation from a short segment of cord was abolished. Pertinent to this point are three cases of poliomyelitis in monkeys which have come to our attention, in which no paralysis was observed following inoculation by either the intranasal route (2 cases), or by skin inoculation (1 case). All three animals showed a definite febrile response within about a week following the inoculations and were subsequently examined carefully for signs of paralysis. Two animals were killed 10 days after the febrile response, the third 3½ months after the febrile response. All showed typical poliomyelitic lesions in the cord, and in one the pathological process was so severe that some sections of the cervical and of the sacral cord showed loss of all anterior horn cells on one side. Brief protocols of these cases may now be considered:

Case A 89. This animal was inoculated intranasally on one occasion with 1 cc. of suspension of stool from a chimpanzee (A 83) which had poliomyelitis (Howe and Bodian, '41). Other rhesus monkeys inoculated with this material had succumbed to typical paralytic poliomyelitis. Animal A 89 showed a typical febrile response (106°F.) 4 days after the inoculation, but the temperature returned to a normal baseline after a few days. No other clinical signs developed during the period of two weeks following inoculation, and careful examination revealed no muscular weakness. The animal was killed, and serial histological sections prepared of the olfactory bulbs, brain, and spinal cord. Typical severe lesions of poliomyelitis were present in the olfactory bulbs; the rest of the brain

showed the distribution of lesions characteristic of intranasally inoculated animals (Bodian and Howe, '40) as far caudally as the medulla oblongata, but no lesions were found in the spinal cord. Lesions were numerous and severe in rostral parts of the brain, and diminished in severity and number in the caudal direction, until in the region of the medulla oblongata only a few small lesions were present (Plate 1). In *summary*, it may be stated that in this case the pathological process of poliomyelitis, initially severe in the centers first invaded by virus, had diminished in severity as spread of virus had occurred, and had finally become spontaneously arrested before any of the ordinarily highly susceptible motor centers of the medulla or spinal cord had become involved. If the arrest in this case was due to the smallness of the dose of invading virus, this dose was nevertheless capable of producing a severe degree of pathological change in the olfactory bulbs as well as in the rostral brain centers ordinarily involved after intranasal inoculation.

Case A 172. This animal was inoculated intranasally on 5 successive days with a suspension of stool obtained from a human case of poliomyelitis. On the eighth day following the first inoculation, a typical febrile response occurred, which lasted for 3 days. However, no paralysis and no other signs of poliomyelitis were observed during the next 2 weeks. The animal was killed 3 weeks after the first inoculation. Serial sections of the brain, including both olfactory bulbs, showed typical, severe lesions of poliomyelitis, with a distribution of lesions characteristic of intranasally inoculated animals (Bodian and Howe, '40). In the spinal cord extensive and severe lesions of poliomyelitis were present in the cervical and sacral regions, with lighter involvement of the lumbar cord. In some sections of the cervical and sacral cord all of the anterior horn cells on one side were destroyed and phagocytized. In *summary*, it appears that in this case an attack of poliomyelitis had occurred, and although a characteristic encephalitis was present, as well as severe damage and destruction of many anterior horn cells, *no clinically demonstrable paralysis* was present.

Case 866. This animal was inoculated on three occasions (Nov. 4, Nov. 16, and Nov. 22, 1938) by an intracutaneous injection of Wfd virus (Trask and Paul, '36). Although a small temperature rise of one degree occurred 10 days following the first inoculation, it was not considered significant at the time, and the animal was allowed to survive for 3½ months. During this time no muscular weakness or other signs of poliomyelitis were observed, and the animal was active and in excellent condition. Serial sections of the olfactory bulbs later were found to be normal, but a considerable number of residual lesions of poliomyelitis was found in many of the brain centers usually involved after intracutaneous inoculation, as far rostrally as the midbrain tegmentum in the brain stem, and in the motor cortex (Bodian and Howe, '40). In the spinal cord numerous chronic lesions were also found, and in the lumbar cord numerous anterior horn cells had been destroyed. Nevertheless, at a distance of 1 or 2 mm. from a region of cord which

showed perivascular cuffing, loss of nerve cells, and glial infiltrations, sections often showed no pathological changes whatever.

These three animals illustrate the fact that in some cases of poliomyelitis in which a fully developed pathological picture of encephalitis and myelitis is present, *no clinically demonstrable paralysis may be detected*. In such cases of non-paralytic poliomyelitis, the fact of the absence of paralysis may be merely accidental and determined by the distribution of the destroyed anterior horn or other motor cells, even though large numbers of such cells may be affected. It appears, then, that arrest of the pathological process can occur in a very late stage, when subclinical paralysis is already present. This spontaneous arrest after the most susceptible regions of the nervous system, the anterior horns, have been severely damaged, but before clinically observable paralysis has occurred, is difficult to explain except on the basis of the operation of unknown constitutional factors, since it seems inconceivable that the immunity of the uninvolved nerve cells could have developed secondary to the infection itself in such a short time (Sabin, '40). The observations made in these cases are encouraging in that they suggest that when the pre-existing factors of host resistance are better understood, they may be made to operate effectively in preventing paralysis, even after the disease has destroyed many nerve cells. Furthermore, we concur with Sabin and Ward ('41) in the belief that "the transitory character of the paralysis in some monkeys may depend in part on the fact that apparently normal function can be carried on with less than the normal number of nerve cells and in part on the probable, but not proved, possibility that not all nerve cells attacked by poliomyelitis virus are irreversibly damaged." As a matter of fact, a high degree of probability as to the reversibility of changes in nerve cells in poliomyelitis has been lent by experimental evidence presented in another connection (Howe and Bodian, '41), which shows that nerve cells exhibiting extensive chromatolysis may subsequently regain normality.

DISCUSSION

The recent description by Sabin and Ward ('41) of non-paralytic poliomyelitis in rhesus monkeys is of great interest in connection with our own findings. These workers used human virus or first-passage

virus, and it is well to note that most of our cases of non-paralytic poliomyelitis were produced in the chimpanzee and rhesus monkey by means of inoculation with untreated human or chimpanzee stools. Our observations confirm those of Sabin and Ward with respect to the finding that non-paralytic poliomyelitis may exist in the experimental animal, though severe lesions may be present in the cord, and that there are no reliable clinical or laboratory criteria for the diagnosis of non-paralytic poliomyelitis. However, in other points we cannot subscribe to their conclusions. First of all, as we have shown, although non-paralytic infection may be associated with severe neuronal damage in the spinal cord, such cord changes, or in fact any cord changes whatever, need not be present. *The virus of poliomyelitis is capable of producing an encephalitis, with or without associated clinical symptoms, in the absence of any pathological change in the spinal cord.* This encephalitis is typical of the disease only insofar as the distribution of lesions is concerned, and as we have previously shown (Bodian and Howe, '40; Howe and Bodian, '41), this distribution depends on the differential susceptibilities of cerebral centers to the virus of poliomyelitis, and to the portal of entry of the virus. The statement of Sabin and Ward that "there is as yet no evidence that there exist strains of poliomyelitis virus without the cardinal property of producing neuronal lesions in the spinal cord and distinct flaccid paralysis," is correct only when qualified by the statement that in any particular animal the virus may set up a characteristic encephalitis, without invading the cord. These workers, in fact, mention a single case in which they failed to find lesions in the cord though sections from six levels were examined, yet lesions were present in the medulla and virus was obtained from this region of the brain. It is clear enough that the pathological diagnosis of poliomyelitis can be made by the finding of neuronal destruction in the cord, coupled with glial infiltration and perivascular cuffing, but in the absence of such findings, the diagnosis can also be made by an adequate determination in serial sections of the distribution of characteristic lesions in the brain.

SUMMARY

1. Evidence is presented from findings in chimpanzees and rhesus monkeys, that the disease of the nervous system produced by the

virus of poliomyelitis may be spontaneously arrested at any stage after the virus has entered the nervous system. This arrest may take place preceding any paralysis, thus producing a "non-paralytic" attack in which lesions are present in the central nervous system, or it may occur after the onset of paralysis.

2. The minimal degree of pathological change observed in non-paralytic cases is a regional destruction of neurones, associated with characteristic mesodermal-glial infiltrations and perivascular cuffing, at and near the site of entry of the virus into the central nervous system. The spinal cord need not be involved, nor is it invariable for a full development of the pattern of distribution of cerebral lesions to occur. Spontaneous arrest may take place, after intranasal inoculation, before the cord is involved, but after a severe and typical encephalitis rostral to the medulla oblongata has developed. On the contrary, cord changes without lesions in susceptible centers of the brain have never been observed.

3. The maximal degree of pathological change observed in non-paralytic cases is practically equivalent in distribution and severity to the pathological involvement which one finds in paralytic cases. The absence of observable paralysis in many cases is due to the fact that, although many nerve cells are destroyed in the spinal cord, the distribution of the destroyed motor neurones is too scattered to involve a single functional muscle group sufficiently to produce clinically evident functional loss. In such cases, nevertheless, one may find all the anterior horn cells destroyed in one microscopic section, whereas sections as close as 1 mm. may be unaffected.

4. It is doubtful whether virus entering the central nervous system in smaller quantity than the minimal infective, or pathogenetic, dose is capable of multiplying or spreading to any considerable degree before being sterilized locally. The minimal infective dose, of course, may be determined by constitutional factors of the host or by varying susceptibilities of the nerve centers first invaded.

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PLATE I

NON-PARALYTIC, ARRESTED POLIOMYELITIS AFTER INTRANASAL INOCULATION IN THE RHESUS MONKEY (A 89), WITH SPONTANEOUS ARREST OF THE PATHOLOGICAL PROCESS BEFORE THE SPINAL CORD HAD BECOME INVOLVED, AND MINIMAL INVOLVEMENT OF THE MEDULLA OBLONGATA

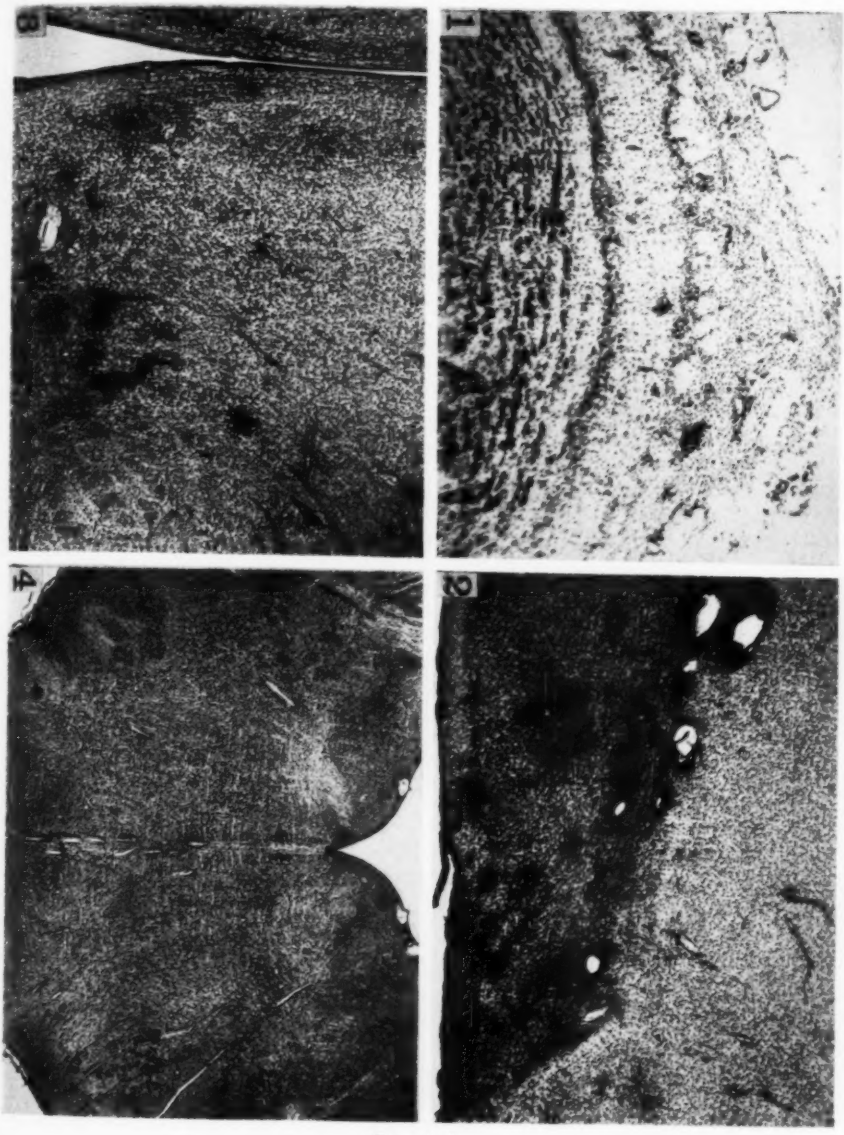
FIG. 1. Olfactory bulb, ×45.

FIG. 2. Region of anterior olfactory nucleus, ×20.

FIG. 3. Hypothalamus, ×20.

FIG. 4. Medulla oblongata at level of facial nucleus, ×10.

Note the severe reaction in the olfactory bulb, anterior olfactory nucleus and hypothalamus, and the absence of lesions in the section of the medulla oblongata. No lesions were present in the spinal cord (Gallocyanin stain).



FIGS. 1–4

UNIV. OF MICH.

POLIOMYELITIS IN THE CHIMPANZEE: A CLINICAL-PATHOLOGICAL STUDY[1]

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The search for the portal of entry of virus in human poliomyelitis has made it imperative to survey the possibilities among other primates which are susceptible to this disease. It is now clearly apparent that host variabilities render suspect any generalities concerning portals of entry in man which are derived from experiments on rhesus monkeys. In this animal intact skin and mucous membranes bar access of virus to the central nervous system at every point except the olfactory area. This fact has been attested by various observers and also in our own laboratory where not a single infection has resulted in 27 experiments involving complete bilateral section of the olfactory tracts. At the same time, reports indicate that other types of old world monkeys, especially the cynomolgus macaque (Macaca irus), may be susceptible to virus introduced by feeding, by pharyngeal swabbing, or by stomach tube (1–6). Since factors of host variability are thus definitely indicated, it was thought especially important to study the reactions of a primate as close as possible to man. The chimpanzee was selected for this purpose not only because of its availability, but also because it is reported to be susceptible to poliomyelitis by natural contagion. Two animals in a children's zoo at Cologne, Germany, suddenly exhibited a flaccid paralysis during a poliomyelitis epidemic. One subsequently died and the spinal cord is said to have contained lesions typical of this disease (7). It is difficult to evaluate such a finding, but the suggestion is strong that the

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chimpanzee possesses a much greater susceptibility to poliomyelitis than does the monkey.

This paper is concerned with six chimpanzees, five of them in excellent condition, which were infected in pairs by three different portals, the olfactory, the oral and the gastrointestinal. Some of the findings on four of the animals have already been reported in preliminary form (8) and will be recounted here in greater detail. It has been previously pointed out (5, 9) that passage strains of virus may exhibit more restricted potentialities in a host to which they have become adapted than do recently isolated ones. For this reason it was regarded as particularly important in experiments involving portals of entry to work with unchanged virus. Accordingly, untreated stools of poliomyelitis patients were used as a source of virus. The details involved in testing the potency of these inocula have been reported elsewhere (10). Suffice it to say here that a pool of stools from 6 to 7 different patients was used for all the experiments. Each stool had previously been shown to contain virus by intranasal inoculation into a rhesus monkey. The stools were preserved in glass ampules kept at the temperature of dry ice and, although the chimpanzee experiments covered more than a year, no diminution in the potency of the inoculum could be attributed to the passage of time. The fact that one of the most potent stools included in the pool was exhausted before the sixth and last experiment, resulted in a slight reduction of potency of the pool as judged by intranasal inoculation into the rhesus monkey, but all control observations pointed to its constancy during the other experiments.

It is of great importance to note that although the infective material used produced prostrating paralysis or death in all rhesus controls, the disease in the chimpanzees was milder in all but one case, and clinically was identical with that commonly seen in man. A description of the experiments themselves is best given by brief protocols of the individual cases.

METHODS AND MATERIAL

All chimpanzees were killed during the acute stage of the disease by bleeding under ether anesthesia. After removal of material for virus assay, they were then perfused through the aorta with about 200 cc. of normal salt solution followed by 2–3 liters of 10 per cent formalin, containing 1 per cent acetic acid. The follow-

ing material was removed in all cases for histological examination after staining with the gallocyanin method of Einarson (11): The brain with olfactory bulbs was removed and prepared for section together with the spinal cord, sympathetic chains and coeliac ganglia, cervical and lumbar spinal ganglia, ciliary, sphenopalatine, Gasserian, vagal, and glossopharyngeal ganglia, intestinal tract, and salivery glands. The brain stem and representative blocks of the cerebral cortex were embedded in celloidin, cut serially at 60 micra, and every twentieth section stained with gallocyanin. The olfactory bulbs, peripheral ganglia, and representative blocks of spinal cord were sectioned serially at 10 micra. In most instances all sections were stained. In some cases, sections were prepared of parts of the intestinal tract and salivary glands, and of the geniculate ganglia.

INTRANASAL INOCULATIONS

Case A83. Dec. 20, 1939. Brunhilda, a 4 to 5 year old female chimpanzee was inoculated in each nostril with 1 cc. of untreated supernatant fluid from a pool of seven different human stools of demonstrated potency. The animal was deeply anesthetized with nembutal and placed on its back with head dependent. A plug of cotton was inserted into the nasopharynx to prevent swallowing of the inoculum. The stool was gently instilled into each nostril and the head was maintained in the dependent position for fifteen minutes. The cotton plug was then removed and the animal left flat on its back for another thirty minutes. During this time any stool which had run down into the pharynx was removed with a cotton swab. The procedure was repeated on two successive days. On one occasion a slight abrasion of the posterior pharyngeal wall was noted. Rhesus A84 was inoculated intranasally as a control and was paralysed on Jan. 1.

Dec. 25–26. The rectal temperature became elevated from a preinoculation level of 102° to a maximum of 104.4°F.

Dec. 27. Temperature 101.8°. No other changes.

Dec. 28. Temperature 98.6°. There was a complete flaccid paralysis of the left hand, arm, and shoulder. No reflexes were obtainable. The right hand and arm were reduced in strength, and biceps and triceps reflexes could not be obtained, although the periosteal radial and ulnar reflexes were present. The legs appeared to be normal in strength and knee kicks and ankle jerks were readily obtained. Cranial nerves were normal. The animal was listless and lay on the table without restraint, eyes closed except when disturbed. Lumbar puncture revealed turbid spinal fluid which clotted too rapidly for counting. Sections of the clot showed many white cells, the majority lymphocytes. The animal was etherized, bled, and the cervical enlargement of the spinal cord removed with sterile precautions for virus studies. Some stool was also expressed from the rectum. An emulsion of the cervical cord produced poliomyelitis typical both clinically and pathologically, in 2 rhesus monkeys (A87 and A204) and in one chimpanzee (A174). The stool of A83 proved to be extremely potent and subsequently produced typical severe poliomyelitis on intranasal inoculation in 13 of 15 rhesus monkeys.

Microscopic findings: The distribution of lesions in the central nervous system was identical with that seen after severe intranasal infections in the rhesus monkey (12). The olfactory bulbs showed maximal invasion with heavy perivascular cuffing, neuronophagia of mitral cells and severe glial reaction in all layers of the bulbs except the central granular layer (Plate 1, fig. 1). The brain showed an extensive meningitis. Lesions of grey matter were exceedingly severe and consisted of a continuous line of perivascular cuffing and infiltrative foci extending through the entire brain stem from the anterior olfactory nucleus (Plate 1, figs. 2–4) to the spinal cord. As in the rhesus monkey, the chief reaction involved the secondary olfactory nuclei (anterior olfactory nucleus, tuberculum olfactorium, parolfactory areas, amygdala, and hippocampus), the nucleus of the diagonal band, the preoptic area, the hypothalamus, the large cells of the reticular formation of the midbrain and hindbrain, and the vestibular nuclei. There were also extensions of lesions to the midline nuclei of the thalamus, the globus pallidus, the substantia innominata of Reichert, the claustrum, and the habenula, as well as to the precentral cortex and lateral thalamus. A few lesions were also present in the putamen and caudate nuclei. Associated with the changes in the vestibular nuclei were heavy lesions in the roof nuclei of the cerebellum and the cortex of the cerebellar vermis. There was also "cuffing," leucocytic infiltration, and neuronophagia in the motor nuclei of the third, fifth, and seventh cranial nerves, while cuffing and infiltration were present in all the nuclei, both motor and sensory, of the other cranial nerves. The nucleus gracilis and nucleus cuneatus were also involved in the reaction which extended without a break into the spinal cord at C_1–C_3, where there was extensive neuronophagia. The lumbar cord showed some cuffing and infiltration, with beginning chromatolysis in the motor cells of the anterior horn, but no neuronophagia. The Gasserian ganglia and those on the vagus showed neuronophagia and infiltrations which were doubtless secondary to the extensive involvement of the brain, since lesions are also regularly found in these sites in the monkey after intracerebral inoculation. The ciliary and sphenopalatine ganglia were normal. The sympathetic chains, including the superior cervical ganglia, showed scattered lymphocytic foci with some questionable cuffing. This was largely confined to the stellate ganglion and upper thoracic chain. The coeliac ganglia contained several large lymphocytic foci, one of them associated with a heavily cuffed vessel. These findings in the sympathetic ganglia were not surprising considering that large amounts of virus were present in the stools.

Summary of case A83. A chimpanzee which received 3 daily intranasal inoculations of human poliomyelitic stool developed fever on the 5th day and arm paralysis on the 8th day. Examination of the central nervous system showed heavy poliomyelitic lesions involving the olfactory bulbs and olfactory nuclei and forming a continuous series through the brain stem to the spinal cord. Virus was demonstrated in the cervical cord and in the stools (see Tables I and II).

Case A175. Oct. 18, 1940. Adult male chimpanzee "Zombie," severely crippled by cage paralysis (legs useless, arms barely strong enough to swing body over floor, grasp little more than adequate to convey food to the mouth). Under nembutal anaesthesia he was inoculated intranasally with 1 cc. per nostril of the

TABLE I

Distribution of Lesions in Susceptible Centers in the Brains of Chimpanzees

REGIONS OF BRAIN	PORTALS OF ENTRY					
	Oral		Gastro-intestinal		Olfactory	
	A48	A106	A71	A105	A83	A175
Olfactory bulbs					cccc****	cc***
Secondary olf. centers†					cccc****	c***
Nucleus of diagonal band of Broca				c*†	cc**	c**
Preoptic area and hypothalamus		c*	c*	cc**	ccc***	*
Globus pallidus and substantia innominata of Reichert		c	cc*		cccc****	
Motor cortex		c*	cc**	c	cc**	
Lateral thalamus		c**	cc**		ccc***	
Subthalamus and midbrain tegmentum		ccc**	cc**	cc*	ccc***	
Cerebellar cortex					ccc**	
Nuclei fastigii		cc****	c*	c*	cccc****	
Reticular formation		ccc****	ccc***	ccc***	cccc****	
Vestibular nuclei		cc****	ccc****	cc***	cccc****	
III nuc.		cccc***			cccc****	
V nuc. (motor)		c****	ccc***	cc*	cc****	
V nuc. (sensory)	cc*	c*	ccc***	c*	cc***	
VII nuc. (motor)	cc**	ccc****	c	c*	cc****	
IX, X nuc. (sensory)	c	c*	c	cc	cccc***	
X nuc. (motor)		cc***	c	c*	c*	
XII nuc.	c	c*	c	c**	cc**	
Cervical cord		ccc***	cc**	cc***	cc****	

* Neuronophagia or focal infiltration.

c Perivascular cuffing.

† See protocols.

supernatant fluid from a pool of four potent poliomyelitic stools (material had been frozen and thawed two times). Rhesus A176 was inoculated intranasally as a control.

Oct. 22. Temperature was extremely irregular. Had pneumonia, probably tuberculous, but treatment with sulphathiazol was instituted.

Oct. 24. Temperature of control A176 was 106.2°.

Oct. 25. Chimpanzee stool taken on this day negative (clinically and microscopically) in rhesus A177 (ten successive intranasal inoculations).

Oct. 27. Sulphathiazol stopped.

TABLE II

Virus Assay on Chimpanzees

EXPERIMENT	MODE OF INOCULATION	DAYS SINCE LAST INOCULATION	VIRUS ASSAY				
			Spinal cord	Stool	Saliva	Salivary glands	Additional
A83	Intranasal 6 cc. stool	6 (paral.)	+(3)	+(13) −(2)			
A175	Intranasal 2 cc. stool	11 (no paral.)		−(1)			
	6 cc. stool	5 (no paral.)		−(1)			
A106	Oral. Olf. tracts cut 6 cc. stool	9 12 (fever) 14 18 (paral.)	−(1)	+(1) +(1) −(1) +(1)	−(1)		
	6 cc. stool	5		+(1)	−(1)	−(1)	
A48	Oral. 6 cc. stool.	27 28 (fever) 29 (paral.) 33		−(1) −(1) −(1) +?(1)			*33rd day (cont.)* nasal mucosa −(1) tonsil −(2) small int. −(2) large int. −(2)
A105	Gavage. Olf. tracts cut 235 cc. stool	5 (paral.)	+(1)	+(2)			
A71	Gavage 250 cc. stool	9 (paral.)	−(1)	−(4)			

Figures in parentheses represent numbers of test animals.

Oct. 29. Control A176 paralysed, but Zombie showed nothing abnormal, and was accordingly re-inoculated intranasally as above on three successive days, with rhesus A179 as a control.

Nov. 1. Temperature elevated. Chest rales worse; sulphathiazol started again.

Nov. 3. Temperature down. Sulphathiazol stopped; animal loath to swallow and while doing so liquids ran out of the nose.

Nov. 4. Fluids still ran out of the nose. Right side of soft palate seen to sag slightly toward center; uvula pulled slightly left. Temperature climbed rapidly.

Nov. 5. Found dead. Stool negative (clinically and microscopically) in rhesus A181 (ten successive intranasal inoculations). Lungs of A175 were almost completely caseous.

Microscopic findings: The olfactory bulbs contained poliomyelitic lesions, not as severe as in the preceding case (A83), but characteristic, and rather extensive on the right side (Plate 2, fig. 1). The central nervous system contained lesions chiefly in the secondary olfactory centers, mainly on the right side. These were the anterior olfactory nucleus (Plate 2, fig. 2), the olfactory tubercle, and the medial and anterior amygdaloid nuclei. The only other lesions of poliomyelitis were found in the nucleus of the diagonal band of Broca and in the preoptic area (see also Plate 2, fig. 3–4 for absence of lesions in hypothalamus and medulla). The Gasserian ganglia and those on the ninth and tenth nerves were negative for poliomyelitis but contained many cells showing atrophic changes. These were no doubt a part of the "cage paralysis" since the spinal cord showed very marked alterations of this character. The distribution of poliomyelitic lesions in this case is that of a very early preparalytic case, or of an abortive attack. The stools were negative for virus.

Summary of case A175. An adult male chimpanzee suffering from extensive cage paralysis and pulmonary tuberculosis was inoculated intranasally on one occasion with 2 cc. of potent human stool. During the following days the temperature was very irregular and the animal was treated for pneumonia with sulphathiazol. Since he showed nothing on the 11th day when an intranasal rhesus control became paralysed, he was re-inoculated intranasally as above. On the third day following the last inoculation fluids were observed to run out of his nose when he attempted to swallow. Two days later (the 18th day after the first inoculation) he was found dead. Sections revealed typical poliomyelitic lesions in the olfactory bulbs and secondary and tertiary olfactory nuclei. No other poliomyelitic lesions were found in the brain, spinal cord or peripheral ganglia. The stools were negative on two occasions (see Tables I and II).

ORAL INOCULATIONS

Case A106. Feb. 29, 1940. "Teddie" was a robust male chimpanzee weighing 22 pounds, whose age was estimated at 3.5 years. Under nembutal and ether anaesthesia a considerable opening was made in the skull in the right fronto-parietal region, extending slightly across the midline. The dura was incised, and the frontal poles were carefully separated from the olfactory bulbs and allowed to drop back. Thus, both olfactory bulbs and the distal half of each olfactory tract were

clearly visualized. During this procedure no bleeding was encountered and the tracts were cleanly cut without difficulty and without damage to the cortex or olfactory bulbs. Following the operation the animal made an uneventful recovery.

Mar. 5. Given 2 cc. of supernatant fluid from a pool of 6 potent human poliomyelitic stools. The material was taken willingly by the animal on the tongue and lips since there was no sense of smell. About 0.5 cc. was put into each cheek near the entrance to the parotid ducts. Rhesus A115 was inoculated intranasally as a control.

Mar. 15. Rhesus control A115 paralysed.

Mar. 16. Stool taken on this day produced non-paralytic poliomyelitis in rhesus A118 (intranasal-olfactory bulbs positive).

Mar. 19. Temperature elevated from a base line of 101° to 102.8°F. Stool taken on this day produced paralytic poliomyelitis on intranasal inoculation in rhesus A168.

Mar. 21. Stool negative (13 intranasal inoculations into rhesus A136).

Mar. 25. Animal has been listless and had a subnormal temperature for several days. Re-inoculation today and on March 26 and 28. Pre-inoculation stool of this chimpanzee produced non-paralytic poliomyelitis after intranasal inoculation in rhesus A146.

Mar. 31. Right facial paralysis definitely observed, involving mouth and eyelid. Reflex blinking absent on right, but this was not apparent unless animal was threatened. It was recalled that the latter finding was present on March 21st but its significance was not appreciated until the balance of the face became involved.

April 1. Right facial paralysis quite definite. Left arm weak; triceps reflex produces feeble movement suggestive of supination; biceps, periostal radial, and ulnar reflexes hyperactive. Lumbar puncture bloody. Saliva of March 19 and today negative (13 intranasal inoculations into rhesus monkey A119, which showed no clinical signs or lesions in the olfactory bulbs). Saliva of April 1 and 2 was also inoculated intranasally into cynomolgus monkey A143 without reaction.

Apr. 2. No change. Etherized, and bled. Cervical cord, parotid and submaxillary glands, and stool removed for virus studies. Stool was negative for virus when tested intranasally in rhesus A139; but was positive in A169 (non-paralytic infection). Rhesus A138 was inoculated with left parotid gland emulsion by sciatic nerve immersion, but without reaction. This animal subsequently contracted poliomyelitis when the other sciatic nerve was immersed in Rockefeller MV virus emulsion. Cynomolgus A144 received 1 cc. of right parotid gland intracerebrally without reaction, but subsequently contracted poliomyelitis after intranasal inoculation with another strain of virus. Rhesus A209 received 1 cc. of 20 per cent emulsion of right submaxillary gland without reaction. Cervical cord was negative in rhesus A137, as tested by immersing the cut sciatic nerve in cord emulsion. The animal subsequently contracted poliomyelitis after similar treatment of the other sciatic nerve with MV virus.

Autopsy. Upon removal of the brain the olfactory tracts were found cleanly cut. There was a gap of about two millimeters between the cut ends, which had healed smoothly into the pia. It should be noted here that there is no reason to doubt that section of the olfactory tracts completely blocks propagation of virus by the olfactory route. This has been repeatedly demonstrated for the rhesus monkey, in which even immediate post-operative intranasal inoculation is rendered ineffective (4 cases).

Microscopic findings: The olfactory bulbs showed no lesions characteristic of poliomyelitis. There was chromatolysis and degeneration in many mitral cells secondary to the olfactory tract sections, but no perivascular cuffing, focal glial accumulations, or neuronophagia. A slight meningitis was present at the pial surface of the bulbs, as well as over the surface of the rest of the brain (plate 3, fig. 1). No lesions were found in the nucleus olfactorius anterior (Plate 3, fig. 2), tuberculum olfactorium, or in any of the other secondary olfactory centers. The most rostral lesions were encountered in the hypothalamus and consisted of light cuffing and a few focal glial accumulations (Plate 3, fig. 3). From this point caudalward one found lesions of increasing severity in the motor centers of the subthalamus (zona incerta, field of Forel), in the reticular formation of the midbrain (also substantia nigra and nucleus ruber), and in the hindbrain. These reached a maximum in the last region where they were very severe indeed (Plate 3, fig. 4). As in other animals there were extensions of lesions from the hypothalamus to midline thalamic nuclei, from the brain stem to the precentral cortex, and from the latter down to the lateral thalamus; similarly, from the vestibular nuclei to the roof nuclei of the cerebellum. There was extensive cuffing, leucocytic infiltration and neuronophagia in the motor nuclei of the third, fourth, fifth, sixth, seventh, ninth, tenth, and twelfth cranial nerves. This was more prominent on the right side, especially below the level of the tenth nerve, where the preponderance of lesions in the reticular formation was great. The mid-cervical and midthoracic cord showed extensive neuronophagia of anterior horn cells, while in the lumbar cord there was only cuffing and infiltration without apparent cell destruction. Perivascular cuffing and focal infiltrations were found in the mandibular divisions of the fifth nerves and in the portion of the Gasserian ganglia devoted to those divisions. It is possible however that this may have represented a reaction to virus which had migrated out from the hindbrain, and was by chance localized in the mandibular divisions, but in view of the findings in the other animal, A48, to be reported in this group, it seems more likely that virus was moving centripetally along these nerves. Also extremely suggestive was the presence of cuffing and infiltration in the right geniculate ganglion and the right tractus solitarius, which represent points on the visceral afferent pathways from the oral mucosa. There was a questionable infiltrative focus in the left vagus nerve near the medulla. The sphenopalatine and ciliary ganglia were normal. Infiltrations were found in the sympathetic chains, slight in the superior cervical ganglia, and predominating in the stellate ganglia and thoracic portions of the chains. The coelic ganglia

both contained focal infiltrations with cuffing also on the right. The lesions in the coeliac ganglia and sympathetic chains were considered consistent with the presence of virus in the stools.

Summary of case A106. A 3 to 4 year old chimpanzee in which both olfactory tracts had been sectioned was inoculated 5 days later with 2 cc. of human poliomyelitic stool by mouth, on three successive days. Fever was noted on the 12th day after the last inoculation, and right eyelid paralysis on the 17th day. The animal was re-inoculated on the 18th and 21st days, which probably had no effect upon the development of further right facial and left arm paralysis by the 25th day. Histological examination showed no lesions in the olfactory bulbs and olfactory nuclei. The heaviest lesions were present in the medulla. There were also lesions in the mandibular portions of the Gasserian ganglia and in the visceral afferent neurone chain leading from the oral cavity to the medulla. Some lesions were also present in the coeliac ganglia and sympathetic chains, which were presumably secondary to the presence of virus in the stool. Virus was demonstrated in the stool on several occasions but saliva and the right parotid and submaxillary glands were negative. See Tables I and II.

Case A48. Oct. 16, 1939. A four year old male chimpanzee, "Bozo," in robust health, was anaesthetized with nembutal to the point where a stomach tube could be passed without eliciting a gag reflex. By this means he was given 25 cc. of the supernatant fluid from a pool of 6 different potent human poliomyelitic stools. There was no regurgitation and the tube was washed before removal.

Oct. 22. Temperature elevated from a base line of 101–102° to 104.4°F. The animal appeared perfectly well and the temperature was normal the next day.

Oct. 26, 27, 28. Received 25 cc., 25 cc., and 20 cc. of the same stool pool on three days as indicated, by stomach tube. The last two inoculations were done without anaesthesia and on the final one the tube was withdrawn before the entire inoculum had gone in, because the tube became plugged when the animal attempted to regurgitate.

Nov. 16–17. Temperature up from a baseline of 101°, to 103.3°.

Nov. 27. Temperature which had been normal since Nov. 17, reached 102.6° today. Slight nasal congestion.

Daily temperatures were maintained until Jan. 2, 1940, without further change. At no time did the animal show any clinical signs of poliomyelitis.

June 10, 11, 12, 1940. Under chloroform anaesthesia he received 2 cc. of stool pool by mouth on each day indicated above. Half the dose was placed in each cheek, which was gently massaged from without to stimulate facial movement.

At the same time a rhesus control, A149, was inoculated intranasally, along with two cynomolgus monkeys, A143 and A144, which received 9 cc. of stool each by stomach tube. The cynomolgi at no time showed any signs of poliomyelitis.

July 9. Temperature rose from a base line of 101° to 103°. Stool obtained and inoculated intranasally into a rhesus monkey, A162, which showed no clinical signs of poliomyelitis, although non-paralytic poliomyelitis was not excluded in this animal.

July 10. Temperature 106°. Animal listless, but showed no other abnormalities.

July 11. Temperature down to normal. Animal is again active. Lumbar puncture showed clear spinal fluid which clotted immediately. A smear from the clot showed few erythrocytes, and large numbers of lymphocytes. One granulocyte (an eosinophil) was seen. At 4 P.M. ptosis of the left eyelid was noted. Stool inoculated into rhesus A170 produced no clinical or histological reaction (ten intranasal inoculations).

July 12. Ptosis quite marked; animal seen to rub eye frequently.

July 13. Ptosis unchanged. Left lower lip was drawn over teeth when animal yawned or grimaced. No other abnormalities. Lumbar puncture bloody.

July 15. Ptosis less marked, but lip unchanged. Animal anaesthetized, bled, and various tissues removed for virus studies. These resulted as follows: Olfactory area of nasal mucosa negative in rhesus A214. Tonsil negative in rhesus A212 and A213. Walls of small intestine negative in rhesus A217 and A218, large intestine negative in A215 and A216. Stool negative in rhesus A163, and questionably positive microscopically in rhesus A171 (all inoculations done as 10 successive daily intranasal instillations).

Microscopic findings: There was a diffuse meningitis and a few focal accumulations of lymphocytes in the pia-arachnoid of the orbital gyri. Similarly, the olfactory bulbs showed infiltration of lymphocytes around the fila of the olfactory nerve where they pass from the cribriform plate through the subarachnoid space to enter the bulb. There was also perivascular cuffing of some of the vessels which adjoin the fila and penetrate the outermost layer of the bulb. No evidence of actual invasion of the olfactory bulbs could be seen. There was no destruction of mitral cells, no glial foci or cuffing in any portion of the bulbs deeper than the outer layer of olfactory nerve fibers. *No other lesions were present rostral to the medulla.* Reaction was first encountered caudally at the level of the entrance of the fifth nerve and was confined almost exclusively to the left side. Cuffing and focal infiltrations were found in the nucleus sensibilis (Plate 4, fig. 2) and the nucleus of the spinal tract of the left fifth nerve (Plate 4, fig. 3). There was cuffing in the sensory nucleus of the ninth and tenth nerves on the left (also shown in Plate 4, fig. 3). Cuffing, infiltration and neuronophagia were present in the left facial nucleus (Plate 4, fig. 4). The right facial nucleus and right motor nucleus of the fifth nerve showed a little infiltration and cuffing, respectively. There was a lightly cuffed vessel in the right hypoglossal nucleus and one in the right inferior olive. Heavy neuronophagia and lymphocytic infiltrations were

found in the left Gasserian ganglion (Plate 4, fig. 1) while the right ganglion contained one small lymphocytic focus. Two small cuffed vessels were found in the left vagus sheath near the medulla. The right superior cervical ganglion contained a small area of infiltration and cuffing, while the coeliac ganglia showed a few small areas of infiltration and questionable cuffing. The significance of these slight changes is very doubtful. One cuffed vessel was found in the left anterior horn of the cervical cord at C_7. No other lesions were found in the following tissues: ciliary, sphenopalatine, geniculate, nodose, jugular, and submaxillary ganglia sympathetic chains, cervical, thoracic cord and lumbar cord. The salivary glands showed accumulations of lymphocytes of doubtful significance. Despite the presence of many nematode cysts and erosions in the mucosa of the colon, no abnormalities were seen in the cells of Auerbach's and Meissner's plexuses of the jejunum, ileum, colon and rectum.

Summary of case A48. A four year old male chimpanzee reacted to large doses of human poliomyelitic stool by stomach tube, with fever. Eight months later he received 2 cc. of the same stool by mouth on three successive days. On the 28th day following the last inoculation his temperature had risen to 106°F. the next day lumbar puncture showed pleocytosis, and ptosis of the left lid appeared. On the 31st day a definite light left facial paralysis was present. Significant lesions in the nervous system were confined to the left Gasserian ganglion, the left sensory nuclei of the fifth and ninth nerves, and the facial nuclei (see Plate IV). No virus was demonstrated in the nasal mucosa, tonsils, walls of the small and large intestines, or stools (see Tables I and II).

In the case just cited (A48) the disease was so light that the distribution of lesions makes a striking demonstration of the penetration of the virus into the nervous system, via the mucous membranes of the mouth or pharynx. Furthermore, this must have taken place either through intact epithelium or through some insignificant fortuitous break in it. The failure to demonstrate virus in the stools and the questionable character of lesions in the coeliac ganglia is further indication of a limited locus of virus activity. The presence of a few lesions in the right superior cervical ganglion and left vagal ganglion is in no way inconsistent with the idea of an oral-pharyngeal portal. Since the disease was more severe in the other animal of this group (A106), the modus operandi here is less clear. It is very definite, however, that infection could not have taken place via the olfac-

tory portal. In this case the fact of the initial facial paralysis, the predominance of lesions in the medulla, together with the lesions in the Gasserian ganglion and the neural pathways from the mouth and salivary glands points strongly to an oral-pharyngeal portal. There is a possibility however of reinforcement by the gastro-intestinal portal since lesions were also found in the coeliac ganglia, and sympathetic chains. This is consistent with the presence of virus in the stools, and does not necessarily mean that virus penetrated by this route from the intestine as far as the central nervous system.

GASTRO-INTESTINAL INOCULATIONS

Case A105. Feb. 9, 1940. The animal, "Freddie", was a robust male chimpanzee around 3.5 years of age, weighing 21 pounds. Under nembutal anaesthesia supplemented by ether, a generous opening was made in the right fronto-parietal region of the skull extending slightly across the midline. The dura was incised and the frontal poles were separated from the olfactory bulbs and allowed to drop back, thus allowing visualization of the olfactory bulbs and the distal halves of the olfactory tracts. This procedure was accomplished without bleeding, and the olfactory tracts were then cut with sharp scissors and with only a little oozing of blood from a small vessel in each tract. Following the operation the animal made an uneventful recovery.

Feb. 13–17. Received pool of six human poliomyelitic stools by stomach tube. A total of 235 cc. of supernatant fluid was given in 5 daily doses. No anaesthesia was employed and there was no resistance from the animal. The tube was washed out before removal. Rhesus A107 was inoculated intranasally as a control, and was paralysed on Feb. 26.

Feb. 19. Temperature elevated from a base line of 100–101° to 103°; listless.

Feb. 20. Temperature still elevated. Unwilling to move.

Feb. 21. Temperature 103.3°. Had definite weakness of left biceps and flexors or third, fourth and fifth fingers.

Feb. 22. Temperature 102°. Left arm 20 per cent functional, except for index finger and thumb which were still strong. Biceps reflex absent. Triceps reflex very feeble; periosteal radial reflex barely obtainable; periosteal ulnar reflex present but somewhat reduced in activity. The head, neck and other extremities appeared normal. Lumbar puncture showed slightly turbid spinal fluid which clotted on standing. It contained 900 white cells per cubic millimeter, 60 per cent lymphocytes and 40 per cent polymorphs. The animal was etherized, bled and some cord removed from the cervical enlargement for virus studies. Upon intracerebral inoculation into rhesus A110 this cord material reproduced the disease both clinically and microscopically. A stool specimen produced typical poliomyelitis on intranasal inoculation into rhesus A111 and A112.

Microscopic findings: In addition to routine microscopic sections, sections were prepared of stomach, duodenum, jejunum, colon and rectum. All of these sections were stained with gallocyanin, and yielded no positive findings. The olfactory bulbs showed no lesions of poliomyelitis. There was a surface meningitis consistent with a generalized process present elsewhere over the pial surfaces of the brain and spinal cord. Secondary to the olfactory tract section many mitral cells showed chromatolysis and actual degeneration, but nowhere were there present in the bulb tissue any evidence of perivascular cuffing, or lymphocytic and neuroglial foci (Plate 5, fig. 1). Also negative were the secondary olfactory nuclei (Plate 5, fig. 2). The most rostral lesions were encountered in the hypothalamus (Plate 5, fig. 3), in the preoptic region, and in the associated caudal portion of the nucleus of the diagonal band of Broca. There was also a little cuffing in the right motor cortex but the entire thalamus and striatum were normal. More numerous lesions were encountered in the medulla oblongata. Here the heaviest reaction was found in the reticular formation of the caudal portion (Plate 5, fig. 4). Cuffing and neuronophagia were present in the vestibular nuclei, which are the most susceptible centers in the medulla, but the nuclei of several cranial nerves (V motor, VII, X, XII) showed only cuffing and infiltration. The sensory nuclei of the ninth and tenth nerves contained some cuffing also. The cervical and thoracic cord showed extensive neuronophagia and perivascular cuffing. Of the peripheral ganglia, only the coeliac, Gasserian, and those of the vagus were examined. The Gasserian ganglia both showed much cuffing and infiltration, whereas a little questionable cuffing was found in the left vagus ganglion. These lesions were doubtless the result of virus spreading centrifugally from the brain. The coeliac ganglia showed definite cuffing and infiltration which was entirely consistent with the stomach tube inoculation and the presence of virus in the stools.

Summary of case A105. A three and one-half year male chimpanzee in which both olfactory tracts had been sectioned was inoculated four days later with 235 cc. of potent human poliomyelitic stool in 5 daily doses by stomach tube. On the 6th day the temperature rose from a base line of 100–101° to 103°. The animal was listless and continued to have fever until the 8th day when weakness of the left arm was noted. On the 9th day there was a complete left arm paralysis. Typical poliomyelitis was produced in rhesus monkeys by inoculations of cervical cord and stool. Microscopic examination of the brain showed typical and extensive lesions in the cervical cord and medulla. The lesions diminished in severity in the rostral portions of the brain stem, none being found rostral to the preoptic area and caudal portion of the associated nucleus of the diagonal band of Broca. The olfactory nuclei showed no evidence of invasion. The presence of infiltration and cuffing in the coeliac ganglia suggested the sympathetic

nerves as the most probable route for virus spread from the gut to the central nervous system (see Tables I and II).

Case 71. Nov. 20–25, 1939. The animal, a male chimpanzee, "Willie," weighing 27 pounds, age estimated at 4 years, received a total of 250 cc. of supernatant fluid from a pool of six potent human poliomyelitic stools. The inoculum was administered under nembutal anaesthesia by stomach tube in equal doses on five different days. There was no struggling or regurgitation and the tube was washed out before removal. Two rhesus monkeys A72 and A73 were inoculated intranasally with the same material.

Nov. 28. Control A73 prostate.

Nov. 29. Temperature elevated from a base line of around 101° to 103.4°.

Nov. 30. Temperature still up. Control A72 prostate.

Dec. 3. Temperature, gradually declining since Nov. 30th, reached 99.4°. Animal not overtly sick.

Dec. 4. Animal found with an almost complete left arm paralysis. Shoulder girdle and upper arm without function. Biceps and triceps reflexes absent, though easily obtained in the right arm. Fingers showed about 10 per cent of normal power of flexion. Periosteal radial, and periosteal ulnar reflexes practically absent, though strong and active on the right. No other abnormalities were noted. The animal was etherized and bled. Cord was removed from the cervical enlargement for virus studies and was negative on intracerebral inoculation into rhesus A82. Stool removed from the rectum did not produce clinical poliomyelitis on intranasal inoculation into rhesus A80 and A81, and A210 and A211. The first two animals later contracted poliomyelitis after inoculation with Rockefeller MV virus, while the latter two showed no lesions in their olfactory bulbs or spinal cords.

Microscopic findings: In addition to routine sections, sections were prepared of stomach, duodenum, jejunum, ileum, colon and rectum. All were stained with gallocyanin, but yielded no positive findings. The olfactory bulbs showed a surface meningitis as a part of a widespread pial reaction involving most of the brain surface. There were no lesions of any sort within the bulbs themselves (Plate 6, fig. 1). Likewise free from lesions were the secondary olfactory centers (Plate 6, fig. 2). The most rostral lesions (cuffing and focal infiltrations) were encountered in the hypothalamus (Plate 6, fig. 3). There was also a slight reaction in the putamen, and more numerous lesions in the globus pallidus, with extension apparently to the motor cortex (areas 4 and 6) and, secondarily, the ventrolateral thalamic nucleus and centre median. More severe reactions were encountered in the midbrain and the recticular formation of the medulla (Plate 6, fig. 4). In the latter, severe reaction was also present in the vestibular nuclei, with extension up to the roof nuclei of the cerebellum. Heavy cuffing and infiltration were seen in the left trigeminal nuclei (both motor and sensory portions). There was a little cuffing in the nuclei of the third and sixth, seventh, tenth and twelfth nerves. The thoracic and lumbar cord showed typical lesions. The sphenopalatine gang-

lia were negative. There was some cuffing and leucocytic infiltration in the Gasserian ganglia and those of the glossopharyngeal and vagus nerves, which had doubtless resulted from the migration of virus centrifugally away from the brain. The coeliac ganglia contained many small areas of lymphocytic infiltration and several cuffed vessels, while the sympathetic chains also showed areas of infiltration and cuffing which were most marked in the lower thoracic region.

Summary of case A71. A four year old male chimpanzee received a total of 250 cc. of potent human poliomyelitic stool by stomach tube. On the ninth day the temperature rose to 103.4°, remained elevated during the tenth day, and gradually declined until the fourteenth day when left arm paralysis was noted. Upon microscopic examination no lesions were found in the olfactory bulbs or related olfactory nuclei. The most rostral lesions were encountered in the hypothalamus. They became progressively heavier in the medulla and spinal cord. There were also significant lesions in the coeliac ganglia and the sympathetic chains, particularly in the lower thoracic levels of the latter. These findings, particularly the former, were thought to suggest the sympathetic nerves as a pathway from the gut to the spinal cord (see Tables I and II).

DISCUSSION

The data presented in the protocols are briefly summarized in Table I, which shows at a glance the essential histological differences encountered in the three types of inoculations. To begin with, the olfactory inoculations were accompanied by extensive lesions in the olfactory bulbs, even though in the second case, A175, the disease was mild. In the more severe case there was involvement of the principal olfactory nuclei, namely the anterior olfactory nucleus, the tuberculum olfactorium, the parolfactory areas, and certain related centers in the temporal lobe, such as the amygdala and hippocampus. There was also an unbroken series of lesions extending caudalward through the brain stem. In contrast, all of the animals receiving stool by mouth or into the stomach showed no lesions in the olfactory bulbs, secondary olfactory nuclei, or temporal lobe. Lesions were very light in the rostral portion of the brain stem and preponderant in the reticular formation of the medulla (not to mention the spinal cord). In the case of the animal A48 which received stool by mouth and suffered only a left facial paralysis, the lesions were so light that

a clear train of inflammation could be made out from the left Gasserian ganglion, through the sensory nuclei of the fifth nerve to the facial nerve nuclei. (The ninth nerve may also have contributed slightly as a route to the brain stem.) Here the portal of entry is unusually clear, but in the other animal which received an oral inoculation (A106) there is much less in the histological picture to distinguish it from the gastro-intestinal infection. The chief difference relates to the extensiveness of the lesions in the cranial nerve nuclei, especially the motor facial nuclei. It will be recalled that the orally inoculated animals were the only ones in the series to show facial paralysis. One might conclude from these findings that the chimpanzee is quite susceptible to the bulbar form of poliomyelitis (as is man) since these animals received relatively small doses of virus. Furthermore, as in the cynomolgus monkeys which were inoculated by pharyngeal swabbing (4, 5) the localization of paralysis was apparently closely correlated with the portal of entry. It is regrettable that in these latter observations the possibility of olfactory infection was not excluded by histological examination of the olfactory bulbs. This means that definite statements regarding the pharyngeal portal can be made only for the chimpanzee, but not without some reservation because of the smallness of the series of animals. One is struck, however, with the difference between the chimpanzee and the rhesus monkey in this respect. The latter animal, which can be infected through the pharynx only by the direct injection of virus into the tonsillar area (13), rarely shows facial paralysis or other bulbar signs when inoculated intranasally. It would appear then that the presence of an initial bulbar paralysis in the chimpanzee is of considerable diagnostic importance as to the portal of entry. Limb paralyses on the other hand could not be correlated with the different localities of inoculation since four out of the six animals showed initial left arm paralysis.

As regards the ease with which the chimpanzee can be infected by the gastro-intestinal route it again differs strikingly from some of the monkeys. It is significant that at the time the chimpanzee experiments were in progress the same stool pool was given repeatedly by stomach tube to a vervet guenon, a baboon, two cynomolgus monkeys, and one 3 months old rhesus. None of these animals showed any

reaction, although the vervet, the baboon and one cynomolgus had no resistance to the same material given later intranasally. While some of these forms, notably the cynomolgus macaque, have been reported susceptible by feeding and gastro-intestinal inoculation the findings have varied considerably in different laboratories and may well be a function of the strains of virus used. It is quite clear that for the particular human strains of virus employed in these experiments, the susceptibility of the lower primates was much less than that of the chimpanzee. Furthermore the fact that the latter animal can be infected through presumably intact mucous membranes should be stressed. The rhesus monkey on the other hand is so refractory by the gastro-intestinal route that six animals failed to show any signs of poliomyelitis when given virus enemas during a bloody diarrhoea which was induced by an 80% alcohol enema given on the previous day.

The findings of virus in the stools of chimpanzees deserves some comment. In the rhesus monkey, which is notoriously insusceptible except by the olfactory route, virus can be demonstrated in the stools only within 48 hours of the time it is given by mouth, but not at the time of paralysis (14–17). Our own experience has been similar. Stools from two rhesus monkeys prostrate from an intracerebral inoculation of MV virus were inoculated intranasally into three animals and stools from four rhesus prostrate after intranasal inoculations of MV virus were inoculated intranasally into 4 other monkeys. None of the test animals developed weakness or paralysis although several showed slight fever. However all of the latter animals were shown either to have histologically normal olfactory bulbs or to be readily susceptible to active MV virus. It would appear, then, that virus is not present in appreciable quantities in the stools of rhesus monkeys which presumably have even swallowed it. These findings stand in contrast to those in the chimpanzees, in which stool virus was readily demonstrated. The animal A83 which received a total of 6 cc. of human stool, had on the first day of paralysis, stool containing enough virus to produce prostrating poliomyelitis in 9 of 10 rhesus monkeys on a single unilateral intranasal inoculation of .5 cc. This stool was obtained on the sixth day following the last inoculation which was deemed ample time for the elimination of the inoculum. Chimpanzee

A105 had strongly positive stools on the first day of paralysis which was the fifth day after he had received 50 cc. of human stool by stomach tube. Here the possibility of incomplete elimination of the inoculum must be considered though it is not very likely. In Chimpanzee A106 which received a total of 5 cc. of human stool by mouth the stools were positive on the ninth day following the last inoculation, the first day of fever (12th day) and the day of paralysis (18th day). These time intervals definitely rule out passage of residues of the original inoculum. The failure to find virus in the saliva or salivary glands of this animal should also be noted. There seemed to be a closer correlation between the severity of the disease and the amount of virus in the stools than with the portal used for inoculation, since with the exception of A71 the two animals most lightly paralysed (A175 and A48) showed the least virus. This correlation has not been made for man, where mild cases are said to show more virus in the stools than severely paralysed patients (18).

There is no doubt then that the chimpanzee presents a larger variety of "natural" portals for the entrance of poliomyelitis virus than does the rhesus monkey and possibly the majority of the other monkeys. However, one must be very guarded in extrapolating any conclusions regarding the chimpanzee to man, despite the fact that the phylogenetic gap is markedly narrowed. The report of poliomyelitis by natural contagion in this animal must be accepted with some reservation and it seems doubtful that poliomyelitis is a disease of chimpanzees as well as of man.

However, in addition to other similarities already noted, the fact that active virus is readily demonstrable in the stools of chimpanzees and man but in the rhesus monkey only for a limited time when it is fed in large quantities seems to place the two former in a category together. When one considers the large number of portals which are presented by the chimpanzee it is suggested that in man, where the susceptibility is undoubtedly greater, the range of portals may be at least as extensive.

SUMMARY

1. Six chimpanzees were inoculated in pairs with human stools containing poliomyelitis virus. Two animals received the inoculum intra-

nasally, two intraorally, and two by stomach tube. In each of the last two pairs one animal had been subjected to bilateral olfactory tract section. All of the animals contracted poliomyelitis.

2. The entire brain stem and considerable portions of the cortex, spinal cord and peripheral nervous system were studied in serial sections.

3. Lesions in the olfactory bulbs and secondary olfactory nuclei were present only in the animals receiving intranasal inoculations.

4. There was a good correlation between the development of bulbar paralysis and intraoral inoculation. One animal of this pair showed definite histological evidence that the virus had reached the central nervous system over the fifth and possibly the ninth nerves.

5. In the animals receiving stomach tube inoculations there was histological evidence that the virus had reached the brain by ascending from the spinal cord.

6. Virus was demonstrated in the stools of the chimpanzees with a regularity roughly comparable to that in man.

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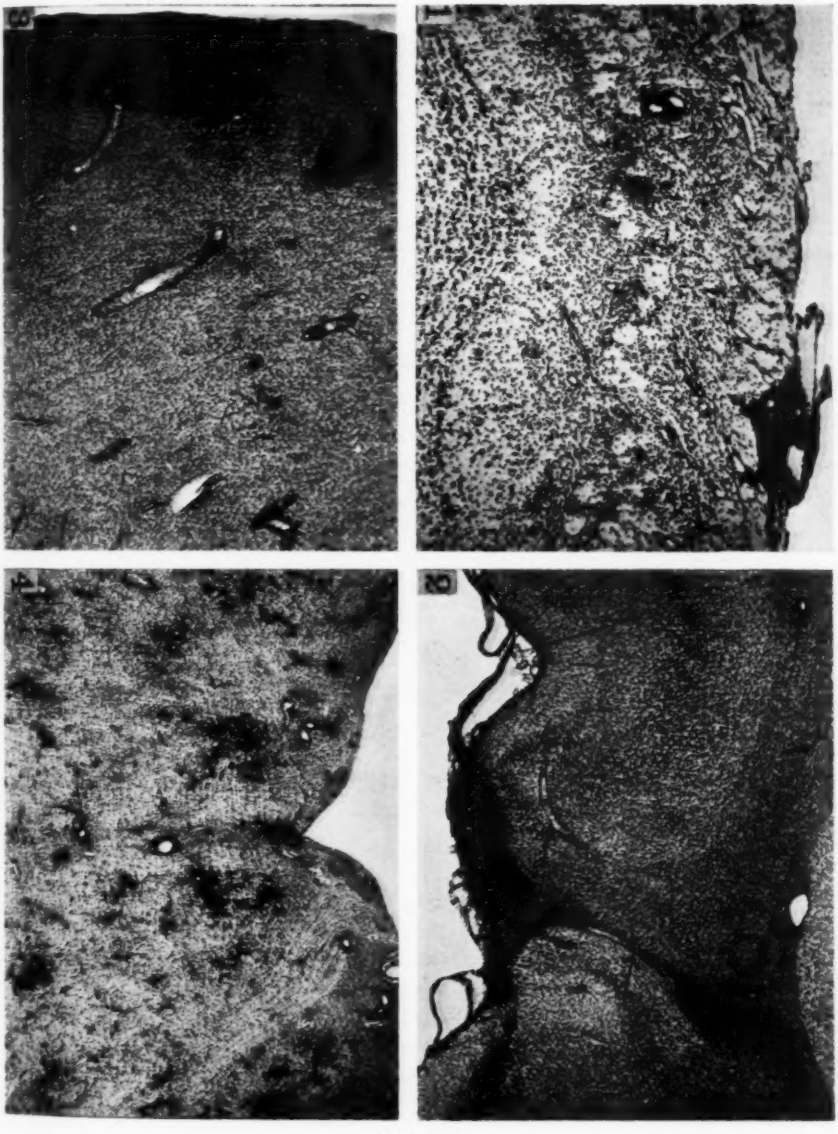
PLATE I

FIG. 1. Olfactory bulb of chimpanzee A83 (inoculated intranasally). Note the dense cellular infiltration, perivascular cuffing and extensive destruction of mitral cells. × 45.

FIG. 2. Region of anterior olfactory nucleus of A83 showing numerous cuffed vessels. × 20.

FIG. 3. Hypothalamus of A83 at the level of the paraventricular nucleus. Extensive perivascular cuffing. × 20.

FIG. 4. Medulla of A83 at the level of the motor seventh nucleus. Note the widespread cuffing and areas of neuronophagia of the nerve cells in the reticular formation and in the lateral vestibular nucleus (upper right). × 20.



FIGS. 1–4

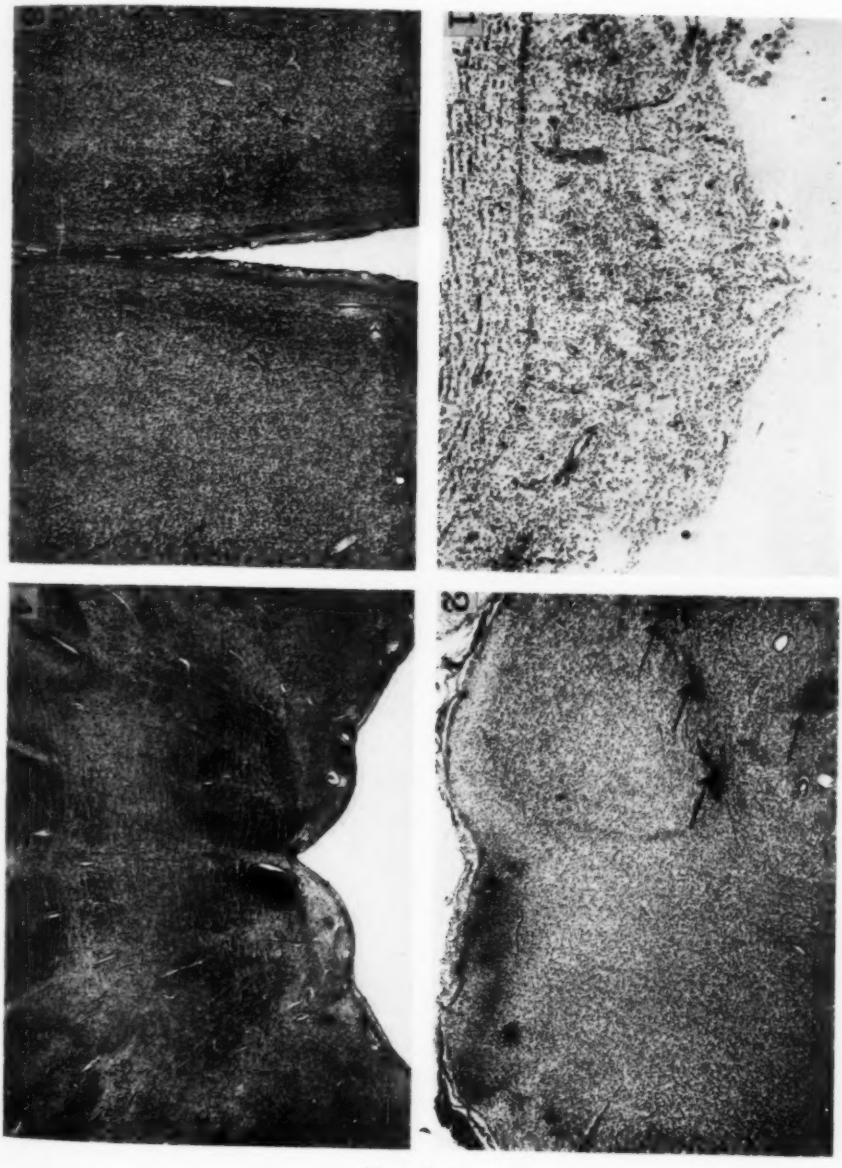
PLATE II

FIG. 1. Olfactory bulb of chimpanzee A175 (inoculated intranasally). Note the dense cellular infiltration, perivascular cuffing, and extensive destruction of mitral cells. × 45.

FIG. 2. Region of anterior olfactory nucleus of A175 showing a focus of lymphocytic infiltration and perivascular cuffing. × 20.

FIG. 3. Hypothalamus of A175 at the level of the periventricular nucleus. No lesions visible. × 20.

FIG. 4. Medulla of A175 at the level of the seventh nerve nucleus. No lesions visible. × 20.



FIGS. 1–4

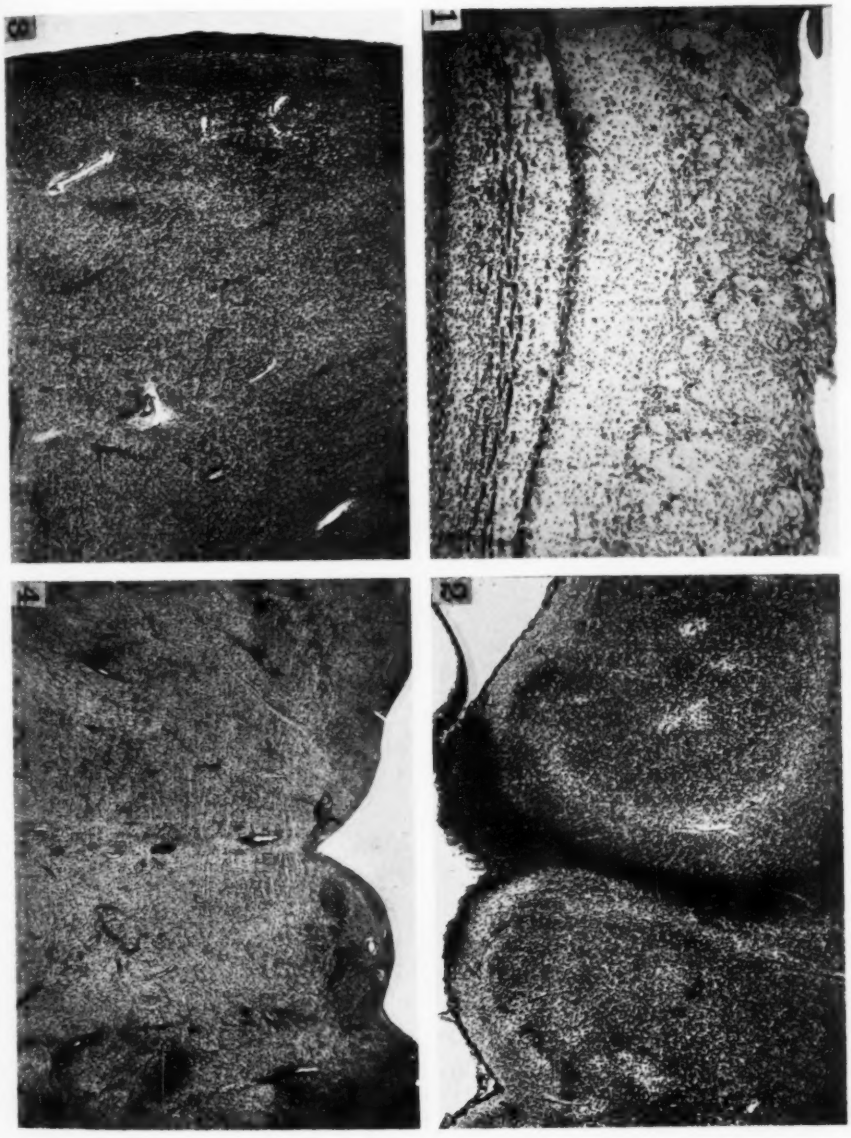
PLATE III

FIG. 1. Olfactory bulb of chimpanzee A106 (inoculated by mouth). There are no visible lesions. The mitral cells are reduced in size because the olfactory tracts had been sectioned one month previously. × 45.

FIG. 2. Region of anterior olfactory nucleus of A106. No visible lesions. × 20.

FIG. 3. Hypothalamus of A106 at the level of the paraventricular nucleus. The most rostral lesions in the brain can be seen as very light perivascular cuffing. × 20.

FIG. 4. Medulla of A106 showing extensive lesions in the right facial nucleus, in the scattered cells of the reticular formation (between facial nuclei and midline) and in the vestibular nuclei (upper right). × 20.



FIGS. 1–4

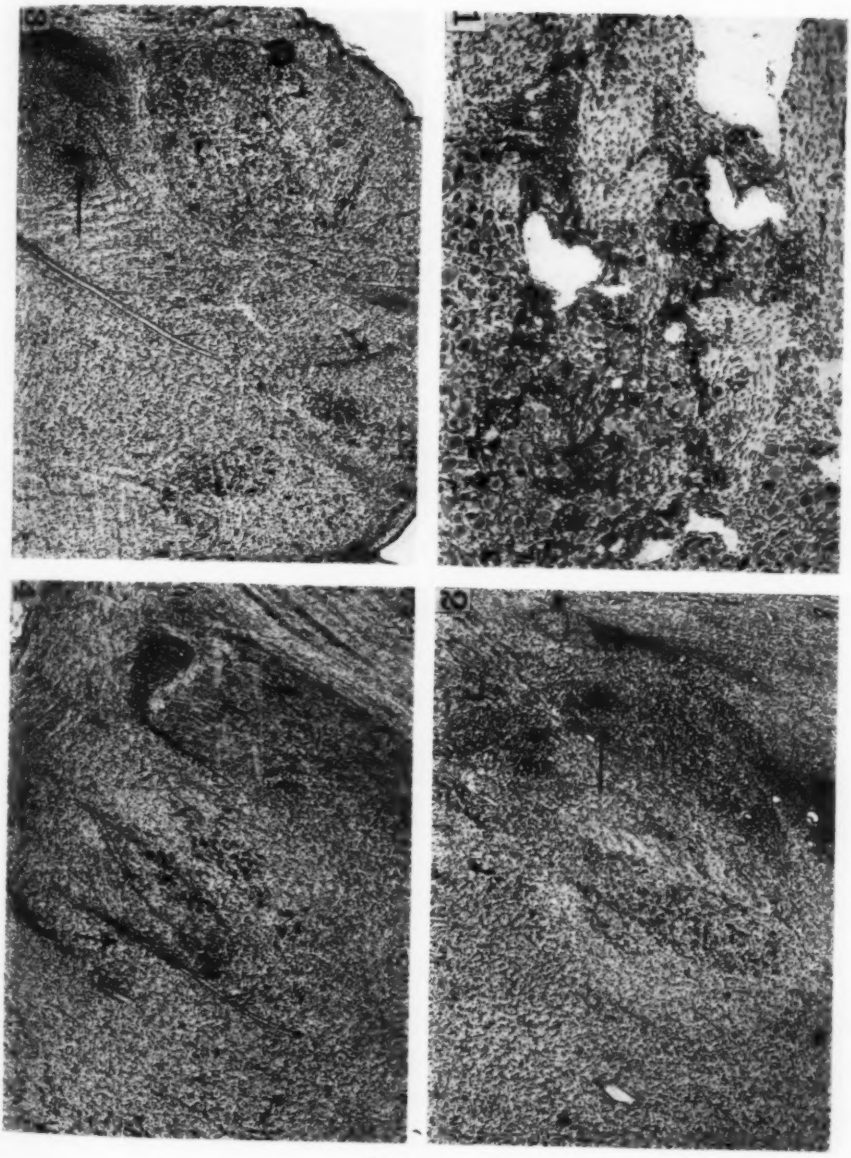
PLATE IV

FIG. 1. Left Gasserian ganglion of chimpanzee A48 (inoculated by mouth) showing extensive losses of nerve cells and lymphocytic infiltration. × 60.

FIG. 2. Nucleus sensibilis of the left fifth nerve of A48 showing cellular focus and cuffed vessel. × 20.

FIG. 3. Spinal nucleus of the left fifth nerve of A48 showing lymphocytic focus. There is also a cuffed vessel in the sensory nucleus of the ninth and tenth nerves (top center). × 20.

FIG. 4. Left facial nucleus of A48 showing neuronophagia and cuffing. × 20.



FIGS. 1–4

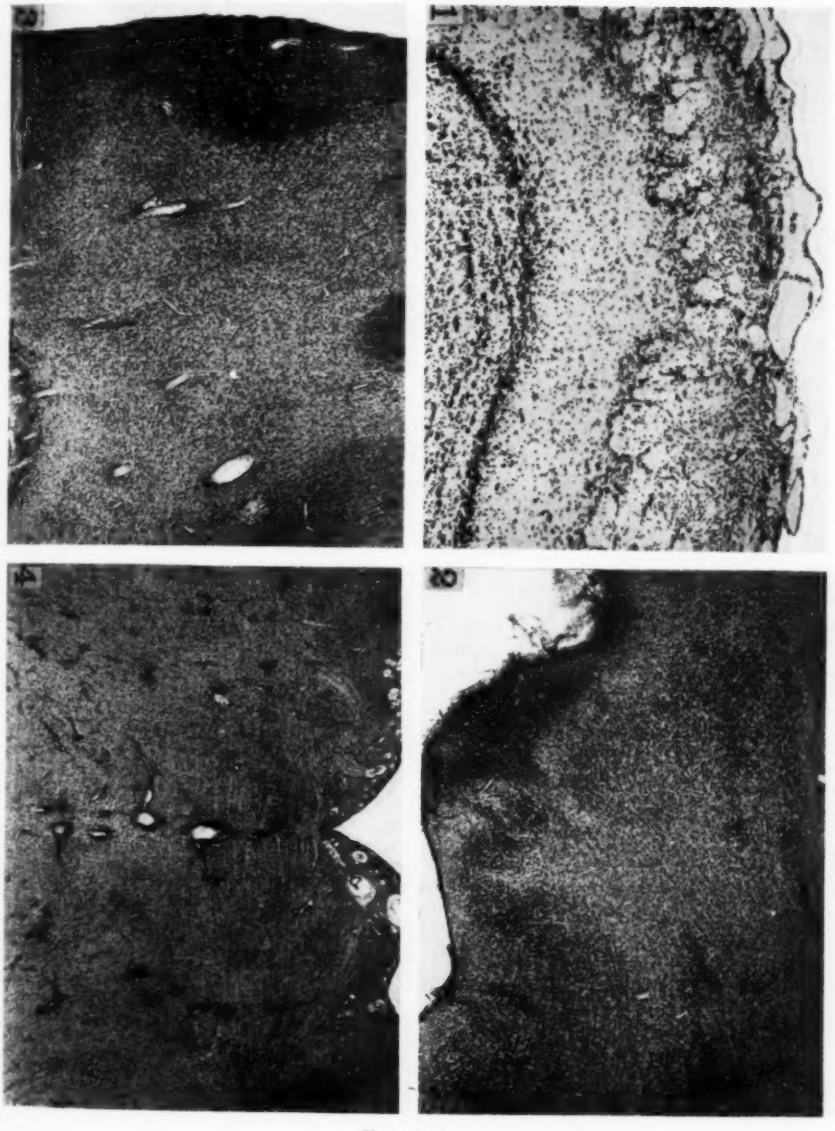
PLATE V

FIG. 1. Olfactory bulb of chimpanzee A105 (inoculated by stomach tube). The bulb is normal except for some changes in the mitral cells resulting from olfactory tract section. Note the slight accumulation of lymphocytes along the fila of the olfactory nerve. This is a part of a light generalized meningitis. × 45.

FIG. 2. Region of anterior olfactory nucleus of A105. No lesions. × 20.

FIG. 3. Hypothalamus of A105 at the level of the paraventricular nucleus, showing light perivascular cuffing. × 20. The most rostral lesions were found in the preoptic [illegible] and caudal part of the nucleus of the diagonal band of Broca.

FIG. 4. Medulla of A105 at the level of the seventh nerve nucleus. There is extensive neuronophagia in the scattered motor cells of the reticular formation. × 20.



FIGS. 1–4

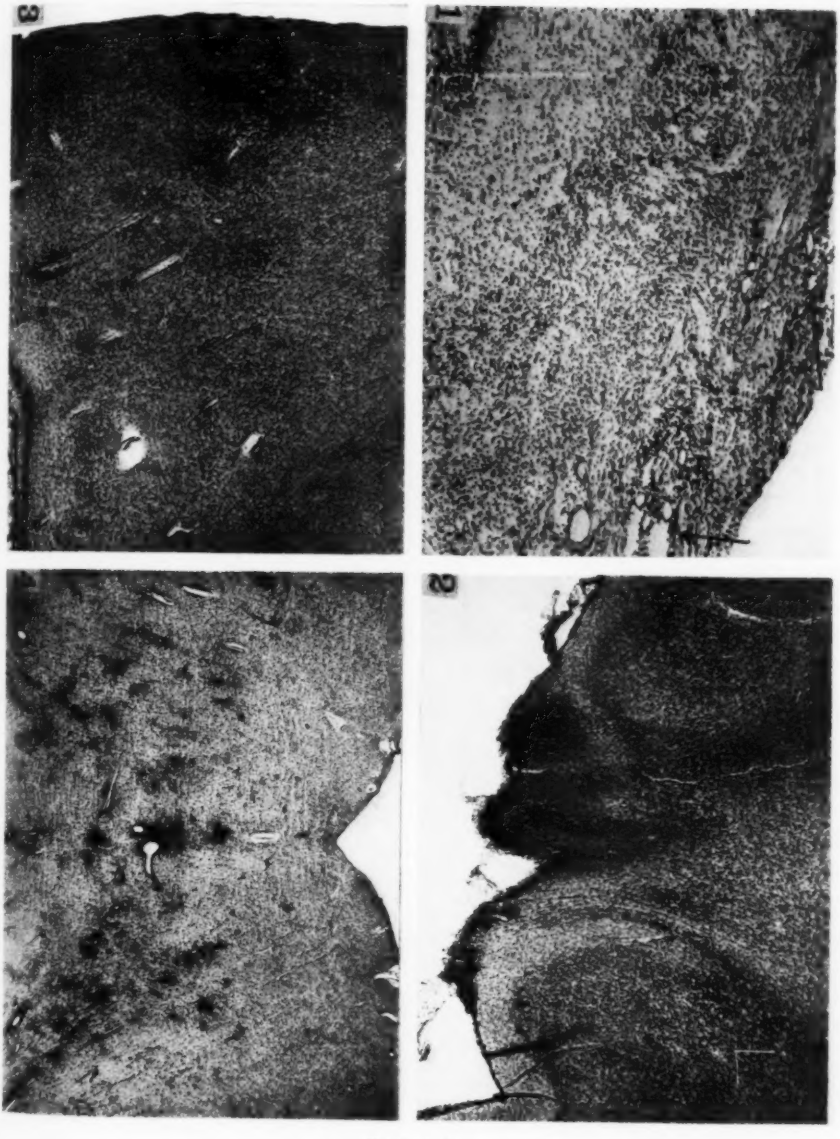
PLATE VI

FIG. 1. Olfactory bulb of chimpanzee A71 (inoculated by stomach tube). No lesions. × 45.

FIG. 2. Region of anterior olfactory nucleus of A71. No lesions. × 20.

FIG. 3. Hypothalamus of A71 at the level of the paraventricular nucleus. The perivascular cuffing represents the most rostral lesions seen in the brain. × 20.

FIG. 4. Medulla of A71 at the level of the motor seventh nucleus. Extensive neuronophagia of the scattered cells of the recticular formation and the vestibular nuclei (upper right). × 20.



FIGS. 1–4

NEUROPATHOLOGICAL EVIDENCE ON THE PORTAL OF ENTRY PROBLEM IN HUMAN POLIOMYELITIS[1]

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It is now well established that the distribution of lesions of poliomyelitis in the brains of monkeys and chimpanzees is, in general, comparable with that described in man (Bodian and Howe, '40; Howe and Bodian, '41b). The study of the pathology of the encephalitis which accompanies human poliomyelitis has in the past, however, not often been primarily directed toward the correlation of the distribution of lesions with the portal of entry of the virus, in the manner demonstrated for the experimental animal. The finding in the experimental animal of marked differences in the quantitative distribution of lesions at certain stages of the disease, as well as differences in the severity of involvement of certain centers after inoculation of virus into various portals, has suggested that a comparative analysis of the distribution of the lesions in human poliomyelitis should lead to a determination of the portal of entry in man, based on pathological evidence alone. The distribution of the lesions in the brains of human cases has been described in more or less detail by various investigators, among whom may be mentioned Harbitz and Scheel ('07), Kino ('28), Marinesco, Manicatide, and State-Draganesco ('29), André-Thomas and Lhermitte ('29), Spielmeyer ('32), Stiefler and Schenk ('33), Környey ('33a), Horanyi-Hechst ('35), Peters ('38), and Swan ('39). Although Harbitz and Scheel early noted the absence of lesions in the olfactory bulbs, also reported by Horanyi-Hechst (15 cases), Harmon and Levine ('38), Graeme-Robertson ('40), Sabin ('40), and others, only Swan has emphasized the absence of lesions in the secondary olfactory

[1] Supported by a grant from the Commonwealth Fund.

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centers, as well as in the olfactory bulbs. Sabin and Ward ('41) have also been unable to demonstrate virus in the olfactory bulbs or the region of the olfactory trigone. Many investigators have now increasingly come to regard the absence of lesions or virus in the olfactory bulbs as important evidence in ruling out the olfactory route as the predominant portal of entry in man. However, additional evidence from other sources would obviously be useful not only in a confirmatory way, but also possibly in determining the actual portals through which the virus invades the nervous system.

It is our purpose here to document the evidence obtained by an analysis of the distribution of the lesions in thirteen human brains, eight of which include complete olfactory bulbs. Uninterrupted serial sections of the olfactory bulbs and brain stems of these brains were prepared, as were also serial sections of large blocks of cerebral cortex from the frontal, parietal, temporal, and occipital lobes. The sections of the brains were cut at 60 micra, and those of the olfactory bulbs at 10 micra, all being stained with gallocyanin so as to permit ready comparison with the similarly prepared experimental material. It is necessary to emphasize that our material was obtained either from rapidly fatal cases of poliomyelitis or from cases in late stages of the acute illness. It is to be expected therefore that the spread of lesions was fully developed, and comparable in that sense with severely paralytic experimental cases. Our hope of obtaining human material in the preparalytic or very early paralytic stage was vain, but one case was remarkable in that death occurred in the acute stage from a cause other than poliomyelitis, namely acute appendicitis.

DESCRIPTION OF CASES[3]

H1. August 21, 1939. H. B., a nine year old white boy complained of a sore throat and had a slight fever.

[3] The writers are greatly indebted to a number of physicians for their cooperation and generosity in contributing this material. In particular we wish to thank Drs. Frank Forry, Amos Michael, Charles Walters, and Lyman Meiks, Indianapolis, Ind.; Dr. George Lyon, Huntington, W. Va.; Drs. J. L. Roark and George Fordham, Montgomery, W. Va.; Dr. C. V. Weller, Ann Arbor, Michigan; Dr. Irving Wohlman, Philadelphia, Pa.; Dr. John Kessel, Los Angeles, Cal.; Drs. Dorothy Anderson and Beryl Paige, New York City; Drs. Edwards A. Park and Sam S. Blackman, Baltimore.

Aug. 27. Headache, muscle pains in back and extremities.

Aug. 28. Clumsy in movement, drowsy, neck stiff.

Aug. 31. Admitted to the Harriet Lane Home, The Johns Hopkins Hospital. Both legs and left arm almost completely paralysed. Rt. arm still had some power at shoulder. Diaphragm and intercostals weak.

Sept. 1. Temperature 40°C.; C.S.F. 111 lymphocytes, Pandy +.

Sept. 2. Completely paraplegic.

Sept. 4. Put into respirator—temperature elevated 4 hours later, aspirated mucous and died. Autopsy was performed within 3 hours, and the entire brain, including both intact olfactory bulbs, was obtained.

Summary of microscopic findings: The pathological changes in the brain of this case which were typical and quite severe, were distributed as indicated in Table I. The rostralmost lesions were found in the gray of the precommissural septum, and among the large cells of the nucleus of the diagonal band of Broca. These centers, as also the large cells of the substantia innominata (nucleus of ansa lenticularis), which appear to be often invaded in human cases, are probably infected by spread of virus forward from the hypothalamus. In the hindbrain, the reticular formation and vestibular nuclei were the seat of severest involvement, with considerable neuronophagia, perivascular cuffing, and intensive mesodermal-glial infiltrations. Of the motor nuclei of the cranial nerves, the fifth, sixth, seventh, tenth and twelfth showed involvement, and only to a moderate degree. The spinal cord lesions were typical and extremely severe in the gray matter of the anterior horns. The distribution of lesions is identical with that seen in the brains of experimental animals following infections which ascended from the spinal cord.

H7. On July 3, 1940, J. H., a 26 year old white male, felt tired, slightly dizzy and anorexic.

July 4. Slight diarrhoea, difficulty in swallowing, and hoarseness.

July 5. Admitted to the Los Angeles General Hospital, Los Angeles, Cal.

July 8. Temp. 102.4°. Swallowing was difficult and thickness of speech was noted. Other cranial nerves were apparently normal. Extremities showed no weakness and reflexes were normal escept for a positive Chaddock's sign on the right.

July 9. Spinal fluid cell count, 790; temperature 102.4°.

July 10, a.m. Developed hiccough. Placed in respirator at 2:45 p.m. After a convulsion lasting 30 seconds, respiration ceased; heart action remained strong until death one hour later. Autopsy performed July 11. There was a diagnosis of terminal bronchopneumonia as well as bulbar poliomyelitis. The brain, lumbar cord and olfactory bulbs, were obtained, but the olfactory bulbs were not intact, there being about one quarter of the right bulb and slightly more than half of the left one.

Summary of microscopic findings: Typical poliomyelitic lesions were found throughout the brain and lumbar cord. These are summarized in Table I. The most significant feature of the pathological findings in the brain of this case,

TABLE I

Distribution of Lesions in the Brains of Human Cases of Poliomyelitis

	H1	H7	H8	H9	H10	H11	H12	H13	H14	H17	H18	H20	H21	INTRANASAL CHIMPANZEE CONTROL A83
Olf. bulbs			Absent		c*		c		Absent		Absent		Absent	ccc***
Ant. olf. nucleus														c*
Olf. tubercle			cc**											ccc***
Pyriform lobe														
Hippocampus														c*
Other olf. nuclei														c
Amygdala			c**											ccc***
Septal area			c**			c	c**	*	c*				c	cc***
Nucleus diag. band of Broca	c**	c**	ccc***		c**		cc**		c*	c*			c*	cc**
Preoptic area	cc*		c*	c*	c*	c*	cc**		c*	*		c*	c*	cc***
Hypothalamus	c**	c**	cc**	c	cc**	c**	cc**	c*	cc**	cc***	cc**	c**	c**	cc***
Globus pallidus	c**	c**	cc**	cc		c*	cc**	c*	c*			c**	c**	ccc***
Substantia innominata	c**	c**	cc**	c		c*	c**		c*	c**	c	c**	c*	ccc***
Caudate & putamen		*	c*	c				c*				*	c	cc*
Frontal pole		c*						c*						c*
Area 4	cc**	c**	ccc***	c**	cc**		cc**	cc**	c**	c***	cc**	c***	cc**	cc**
Other neocortex														
Lateral thalamus	c*	c**	cc**	*	c*		cc**	c**	c*		*	c**	cc**	cc**
Med. & midline thal. nuclei		c**	c**	c*	c*		cc**	c*	c*	c*	c*	c*	c*	cc**
Geniculate nuclei														
Zona incerta	c**		cc**		c**	c**	cc**	cc**	c**	cc**	c**	c*	c**	ccc***
Nuc. Luys		c*	c*	c		c*	*		c*	*	*	c*		ccc***
Field H & tegmentum	cc***	cc**	cc***	c*	cc**	c***	ccc***	c**	ccc***	cc***	cc***	c**	cc**	ccc***
Tectum	cc***	c**	ccc***	c	c**	c***	cc**	c*	cc***	cc***	c**	c***	c**	ccc***
Central gray	cc**	cc**	ccc***	c	cc***	cc***	cc***	c**	cc***	cc***	cc****	c***	cc**	ccc***
Reticular formation	cc***	cc***	ccc***	cc***	cc***	ccc***	ccc***	cc**	ccc***	ccc***	ccc***	c***	cc***	ccc***
Cerebellar hemispheres														
Vermis	cc***	*	cc**	*	c**		cc***	c*	c**	c**	cc**	c**	c**	cc***
Pontine nuclei	*		c*					c		*	*			c*
Inferior olive	c	c*			c*	c*	c*		c*	c*	c*	c**		cc*
Nuclei fastigii	c***	cc***	cc***	c*	c***	c***	cc***	c**	cc**	c****	cc***	c***	cc***	cc***
Dentate nuclei	c	c*	c*	c*	**	c**	c**	c*	cc**	**	c*	c**	c*	cc***
Vestibular nuclei	c***	c****	ccc***	cc***	c***	c***	cc***	c***	cc***	ccc****	ccc***	c****	cc***	cc****
IX & X nuclei	c*	cc****	c*	c	cc**	c**	cc**	c**	c**	cc**	cc****	c*	c*	c**
Other cranial nerve nuclei	c**	cc***	cc**	c*	c**	c***	cc**	c*	cc**	ccc***	cc***	c***	c*	cc***

* Neuronophagia and mesodermal-glial infiltration. c, perivascular cuffing.

which had a short clinical course and in which lesions were absent in the olfactory centers (Plate 1, figs. 1 and 2), was the extremely severe involvement of the nuclei associated with the glossopharyngeal and vagus nerves, and of the reticular formation of the medulla oblongata. Rostral to the motor seventh nucleus, which was also seriously damaged, the intensity of the pathological process was much less marked (Plate 1, fig. 3). In addition to the heavy infiltrations and neuronophagia present in the motor and sensory nuclei of the vagus nerve, including the caudal portion of the nuclei of the tractus solitarius, there was complete bilateral destruction of all the nerve cells in the nucleus ambiguus (Plate 1, fig. 4). This latter finding is of interest because it has never been noted in experimental material and because of the fact that the initial paralysis in this case was bulbar (dysphagia). It seems extremely likely that the portal of entry was the oral-pharyngeal one.

H8. On August 13, F. J., a 6½ year old colored boy complained of a headache and seemed feverish.

Aug. 14. Listless.

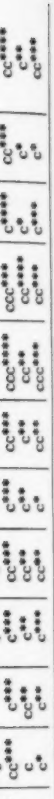
Aug. 15. Asked for drink of water but could not hold the cup.

Aug. 16. Admitted to the Childrens Hospital of Philadelphia. Could not not move head. Pain in back, on flexion of neck. Nearly complete arm paralysis. Absent knee jerks. CSF 540 cells per cu. mm. 96 per cent mononuclears. By evening, breathing was largely diaphragmatic and the child was put into a respirator.

Aug. 17. Nystagmus of left eye—difficulty in swallowing—drowsy.

Aug. 18. Sweating and dyspnea. Still could answer questions and took a small quantity of fluid. Died suddenly. The brain was fixed in formalin, and was practically complete except for the olfactory bulbs.

Summary of microscopic findings: This case was the only one in our series which exhibited lesions in the tuberculum olfactorium and in the amygdaloid nuclei other than the substriatal gray. Although the involvement of secondary olfactory centers was therefore greater than in any other of the cases, it was considerably less than that found in intranasally inoculated chimpanzees (Table I, A83) or monkeys. Unfortunately the olfactory bulbs were not available for histological study, so that a critical determination of the portal of entry in this case is not possible. The distribution of lesions in the brain as a whole was typical of poliomyelitis and as extensive and severe as in any of the other cases in this series (Table I). The possibility exists that the tuberculum olfactorium and amygdala could have been invaded from more caudal centers rather than from the olfactory bulb (see chart 1), although if this were true one must assume that this is not common in ascending infections in man. The presence of lesions in these secondary olfactory centers furthermore demonstrates their susceptibility in man as in the lower primates, and appears to rule out the possibility that these centers can be invaded from the olfactory bulbs without showing pathological change. This is additional evidence that the absence of lesions in secondary olfactory centers in most human cases points toward a non-olfactory portal of entry in most human cases.



* Neuronophagia and mesodermal cellial infiltration. c, perivascular cuffing.

H9. On August 5, 1940, R. D., a thirteen year old white boy of Goshen, Indiana (an epidemic area) was feverish and complained of pain in the back. This latter persisted for two days and then appeared to be improved.

Aug. 8. Left arm and right leg were weak.

Aug. 10. Admitted to the Riley Hospital, Indianapolis, Indiana. At that time he was found to have an extensor paralysis in both arms. The legs were normal. There was a stiff neck. CSF showed 8 cells per cu. mm. and the Pandy test was negative. While in the hospital the boy developed acute appendicitis which resulted in peritonitis and death on August 20, twelve days after the onset of paralysis. There had been no progression of paralysis during this time. The entire brain, including the complete olfactory bulbs and the upper cervical cord were obtained 9 hours after death. The olfactory bulbs which were quite soft, though intact, were fixed in formalin, while the brain was fixed in 1 per cent glacial acetic acid in 10 per cent formalin.

Summary of microscopic findings: The pathological changes in the brain of this case were relatively light, although of characteristic distribution (see Table I), except in the reticular formation below the motor seventh nucleus, and in the vestibular nuclei. These centers showed extremely heavy infiltration of mesodermal-glial elements, neuronophagia, and perivascular cuffing (Plate 2, fig. 4). Rostral to this region the lesions were especially light and extended no farther forward than the preoptic area (Plate 2, figs. 1–3). The cervical cord showed severe damage. The intense involvement of the cord and lower brain stem, with progressive diminution of the number of lesions in the rostral direction is suggestive of an ascending course of invasion of the cerebrospinal axis by the virus, although it was not clear whether cranial or spinal nerves were the primary pathways of virus ingress into the central nervous system.

H10. On August 14, 1940, H. S., a 27 year old male of South Whitely, Indiana (an epidemic area), had a "tired feeling."

Aug. 18. Muscle pains.

Aug. 19. Respiratory difficulty—admitted to the Riley Hospital, Indianapolis, as an emergency. Was put into respirator immediately, but it was noted that right leg could not be moved.

Aug. 20. Unable to swallow—died 5 p.m. The brain and both olfactory bulbs were obtained 6 hours later. A small piece of the lower medulla was preserved in glycerine and some stool was taken from the rectum. The medulla reproduced typical poliomyelitis upon intracerebral inoculation into a rhesus monkey and was carried into the second passage. It produced no growth in blood broth or blood agar and did not cause a fatal encephalitis in 20 mice, 3 rabbits and 5 guinea pigs. The stool also produced poliomyelitis which was typical both clinically and histologically upon intranasal inoculation into a rhesus monkey.

Summary of microscopic findings: This case was of special interest because of the finding of typical poliomyelitic lesions in the left olfactory bulb, the right bulb

being histologically normal. Since this was the only olfactory bulb in our series which showed cuffing, mesodermal-glial infiltrations, and neuronophagia, such as as seen in the bulbs of experimental animals following intranasal inoculation (Plate 3, fig. 1), it was considered especially important in demonstrating that the mode of reaction of the human olfactory bulb to poliomyelitis virus is identical with that observed in the experimental animal. It was remarkable, however, that the distribution of lesions in the rest of the brain of this case was not characteristic of an infection by way of the olfactory portal, since no lesions were found in secondary olfactory centers (Plate 3, fig. 2). The distribution of lesions in the brain, as seen in Table I, as well as the clinical history, suggested a typical ascending infection of the brain similar to the cases with negative olfactory bulbs. The severity of the pathological reaction in the hindbrain (Plate 3, figs. 3 and 4), in addition to the absence of lesions in secondary olfactory centers, seems to rule out the possibility that virus had invaded the brain stem from the olfactory bulbs. In view of the clinical history of dysphagia which suggests the possibility of regurgitation through the nose, it seemed possible that the olfactory bulbs could have infected secondarily by the fortuitous contact of infected oral-pharyngeal secretions with the nasal mucosa. It has been previously shown that virus can invade a previously uninfected portion of the nervous system even in convalescent cases (Howe and Bodian, '41a). The possibility that such a super-imposed infection of the olfactory bulbs may occur during the *acute stage* of an infection produced primarily by invasion through an oral-pharyngeal or intestinal portal, lends a new aspect to both the portal of entry problem and the mechanism of immunity in poliomyelitis. The suggestion may be made that it is not at all unlikely that the lesions in the olfactory bulbs occasionally described in cases of poliomyelitis may be due to such a super-imposed infection, and do not necessarily point to the olfactory portal as the primary point of ingress of the virus to the nervous system. The distribution of lesions in the brains of more of such cases should be determined, however, before ruling out the possibility of primary olfactory infection. In view of the possibilities presented by this case, as well as the considerable mass of evidence in favor of a non-olfactory portal in most human cases of poliomyelitis, it would appear necessary to be critical even of lesions in the olfactory bulbs as an indication of the olfactory portal in man, especially when the clinical history suggests the possibility of superimposed olfactory bulb infection because of regurgitation and dysphagia before or during the acute stage of the disease.

H11. On August 12, 1940, S. R., an 8 year old white girl of Argos, Indiana (an epidemic area), had a headache and felt dizzy.

Aug. 18. Vomited, neck stiff, muscle pains in arms.

Aug. 22. Admitted to the Riley Hospital, Indianapolis, Indiana, in a semicomatose condition. Dysphagia and right facial paralysis were present. No tendon reflexes were obtainable, though there was no obvious limb paralysis. Temperature 106° just prior to death a few hours after admission. The brain and intact

olfactory bulbs were obtained 8 hours post-mortem. Also at autopsy a small stool was removed from the rectum. On inoculation into a rhesus monkey this specimen produced poliomyelitis typical both clinically and microscopically.

Summary of microscopic findings: This case was similar to others in presenting a severe degree of pathological involvement of the reticular formation and cranial nerve centers, especially the right facial nucleus (see Table I), but very slight reaction rostral to the hypothalamus. The most rostral lesions consisted of slight perivascular cuffing in the septal area, all other secondary olfactory centers, as well as the olfactory bulbs, being negative. The case was also interesting in that it was the only one in our series in which lesions were not found in area 4 of Brodmann, although slight perivascular cuffing was observed in the post-central gyrus. The absence of lesions in the lateral thalamus, as well, adds further confirmatory evidence to the finding in the experimental animal that the lateral thalamus is only infected by spread from the motor cortex (Bodian and Howe, '40).

H12. On August 24, 1940, R. S., a 26 year old white male, was admitted to the Long Hospital, Indianapolis, Indiana, as an emergency case. He had been brought from his home in Chirubusco, Indiana, an epidemic area. Admission notes were very meagre but the onset of illness was given as August 17. In the interval before admission there had apparently been a widespread but slowly progressive paralysis, which had finally involved the respiratory muscles. The patient was placed immediately in a respirator without extensive physical examination.

Aug. 25. Irrational; unable to move legs or right arm.

Aug. 26. Died. Entire brain including both olfactory bulbs was obtained within a few hours. The former was fixed in formol acetic and the latter in 10 per cent formalin.

Summary of microscopic findings: Considering the extent and number of lesions, this case and case H8 were the most severe in our series. Plates 4–6 show this distribution and indicate several significantly negative olfactory areas. In addition to severe pathological reaction in regions of the brain stem usually involved, certain centers of less susceptibility, such as the inferior olivary nuclei, showed typical lesions (see Table I). In spite of the extensiveness and intensity of the pathological reaction, however, the olfactory bulbs and secondary olfactory centers, with the exceptions of a single cuffed vessel in the central core of white matter of the left olfactory bulb, and a few infiltrative lesions in the septal area, contained no lesions (Plate 4, figs. 1–2; Plate 6, figs. 1–2). It is almost certain that both the septal area, and the substriatal gray of the amygdaloid complex, which also contained several cuffed vessels and infiltrative foci, were invaded from the hypothalamus and preoptic area, rather than from the olfactory bulb. In view of the severity of the pathological response in this case, it would be most astonishing if the virus had invaded the brain via the olfactory portal without any more pathological reaction than was found in the olfactory bulbs and secondary olfactory centers. It is more likely that a small amount of virus invaded the olfactory bulb from below, perhaps from the septal area, and produced a minimal pathological

response in the region of origin of the olfactory tract in the bulb (Plate 4). Complete serial sections of the olfactory bulbs were otherwise normal in every respect, as far as could be determined. It is also of interest that in spite of heavy involvement of area 4 of the cerebral cortex, lesions were not found in any other part of the cortex. The line of demarcation of the region of the cortex showing lesions, in fact, coincided in a remarkably precise way with the cytoarchitectural borders of area 4 (Plate 5, fig. 1). This is another instance of the special susceptibility of this region of the cortex in poliomyelitis, and of the relatively low susceptibility of other cortical areas. As in other cases there was severe invasion of the vestibular nuclei and their related cerebellar centers (Plate 5, fig. 2).

H13. On August 28, 1940, H. S., a 7 year old colored boy of Powellton, W. Va. (an epidemic area) vomited and complained of headache and stiff neck. There was a positive Kernig sign. CSF cell count was 95 (48 per cent polymorphs), and globulin was present.

Aug. 31. Rt. leg paralysed. During the subsequent two weeks there was gradual extension of paralysis to all the extremities, abdominal muscles and intercostal muscles, so that by Sept. 17th only the diaphragm was still working. There were no bulbar signs however.

Sept. 21. Died suddenly. Autopsy performed 24 hours later after the body had been embalmed. The brain, with both intact olfactory bulbs, and a portion of the first cervical cord segment were obtained.

Summary of microscopic findings: In general, the distribution of lesions in this case was quite widespread, but very few regions of the brain showed severe pathological reaction (Table I). The most rostral lesions were in the septal area, but the olfactory bulbs and other olfactory centers were negative. Although the distribution and character of the lesions were not entirely diagnostic with respect to the portal of entry of virus in this case, it may be supposed that because of the unusually light involvement of the hindbrain it is unlikely that virus had gained access to the nervous system by way of cranial nerves. Nevertheless, it is not impossible that after entrance by way of cranial nerves the point of severest damage may be the spinal cord, with a lighter involvement of centers in the brain. The regional distribution and intensity of lesions in the brain, however, were those which would be characteristic in the experimental animal of cases in which virus had spread rostrally to the brain from the spinal cord, rather than of cases inoculated by the oral-pharyngeal portal or directly into cranial nerves. In many regions in this brain the lesions were in the late reparative stage rather than in the acute infiltrative stage, a fact which may be correlated with the relatively long duration of the acute stage of the disease.

H14. On Sept. 12–13, 1940, E. F., a seven year old white boy was slightly anorexic.

Sept. 14. Pain in neck and chest. Vomited 3 times. Feverish.

Sept. 15. Admitted to Babies Hospital, N. Y. C. Temperature 102.6°.

Pulse, 124; respiration 42. Positive Kernig sign. CFS, 720 cells per cu. mm., with 90 per cent granulocytes—cultures sterile. Rt. biceps reflex hyperactive. By afternoon had become irrational. Was treated with sulphanilimide, since meningitis was suspected.

Sept. 16. Temperature 101°. Respiration 54. Neck stiff and painful—irrational.

Sept. 17. Temperature 104°. CSF, 100 cells, 58 per cent granulocytes. No longer able to move arms—no biceps and triceps reflexes obtainable. Deep reflexes still present in legs, more active on the left. Left chest moved poorly. Rt. lower facial weakness, bilateral nystagmus, and weakness of external recti. Respiration gradually slowed. Patient was placed in a respirator but died at 8 p.m.

The brain and lower half of the spinal cord were obtained, fixed in formalin. The former was complete except for the olfactory bulbs, which were present as fragments only, and small areas of the right gyrus angularis and left inferior frontoparietal region which had been removed for virus studies. Some cervical cord had also been placed in glycerine for virus assay. This cord was subsequently shown to produce no growth in blood broth or blood agar. It was inoculated intracerebrally into 2 rabbits, 7 mice and 2 monkeys. One rabbit and one mouse died within a few hours but none of the other animals showed any signs of invasion of the nervous system. The spinal cords of the two monkeys were subsequently examined histologically without any pathological changes being found. Histological examination of thoracic and lumbar cord from the patient revealed extensive changes in the anterior and posterior horns which were quite typical of poliomyelitis.

Summary of microscopic findings: The distribution of lesions in the brain of this case (Table I) follows the pattern seen in the majority of cases in our series, with no lesions in the secondary olfactory centers and the septal area as the most rostral point in the brain showing pathological change. However, the olfactory bulbs were not available for histological study, so that it is not possible completely to exclude the olfactory route as the portal of entry of virus into the central nervous system. The spread of lesions furthermore does not suggest any of the cranial nerves as pathways of virus ingress into the brain, but resembles most closely the definitive distribution in experimental animals in which virus has invaded the brain from the spinal cord.

H17. On Sept. 4 and 5, 1940, a 23 year old white male farmer of Delphi, Indiana (an epidemic area) had "aching pains and stiffness in the back of the neck, inability to urinate, impaired function of the right leg."

Sept. 6. Admitted to the Long Hospital, Indianapolis, Indiana. Temperature 103°; CSF cell count 214 (88 per cent lymphocytes); blood count revealed 10,300 leucocytes, of which 79 per cent were granulocytes. Abdominal, biceps, triceps, patellar and Achilles reflexes were absent. Unable to flex leg. Acute respiratory distress. Placed in respirator.

Sept. 7. Died after 34 hours in the hospital. The brain and complete olfactory bulbs were obtained fixed in formalin.

Summary of microscopic findings: A severe degree of pathological changes in the brain stem caudal to the preoptic area characterized this case (Table I). Considerable neuronal destruction and numerous infiltrative lesions were present in the subthalamic centers, except the nucleus of Luys, in the reticular formation and central gray, and in all of the cranial nerve nuclei, especially the vestibular, vagal, and hypoglossal groups. The severity of pathological change in the medulla oblongata, and the absence of lesions in the olfactory bulbs and secondary olfactory centers, suggested a non-olfactory and possibly an oral-pharyngeal portal of entry in this case.

H18. On Sept. 8, 1940, R. McD., a 31 year old male of Fairmont, Indiana (an epidemic area) had a headache, muscle pains and fever.

Sept 9. Vomited—began to have respiratory difficulty.

Sept 10. Admitted to Riley Hospital, Indianapolis, Indiana, in extremis—breathing in gasps at long intervals. Was placed immediately in a respirator without physical examination and died ten minutes later. Post-mortem spinal puncture showed 43 white cells per cu.mm. and a trace of globulin. Autopsy was performed three hours after death. The brain, complete except for the olfactory bulbs, was obtained, fixed in formalin.

Summary of microscopic findings: Although the olfactory bulbs were not available for histological study in this case, the absence of lesions in secondary olfactory centers and the general distribution of lesions in the brain (Table I) suggests a non-olfactory portal of entry of the virus into the brain. Especially striking was the severe involvement of the vagal nuclei, comparable in our series only with that in Case H7. Such severe pathological reaction in the vagal nuclei is seen in the experimental animal as a rule only when cranial nerves, especially the vagus, are the pathways of ingress of virus into the brain.

H20. On October 22, S. T., a 32 year old white female, complained of headache, pains in the back, neck and legs and general malaise.

Oct. 24. Admitted to University Hospital, Ann Arbor, Michigan. Neck stiff, vomiting. CSF cell count 900 (67 per cent polymorphonuclears). Both arms weak, no tendon reflexes obtainable, unable to move thighs or knees, though could still move feet. Weakness of intercostal muscles. Put into respirator.

Oct. 26. Vomited repeatedly. Became weaker.

Oct. 27. Died. The brain and both intact olfactory bulbs were obtained and fixed in formalin.

Summary of microscopic findings: The distribution of lesions in the brain of this case was typical in all respects, with moderately severe involvement of the brain stem centers and area 4, and an absence of lesions rostral to the preoptic area (Table I). The pathological picture in the brain was not especially suggestive of an oral-pharyngeal portal, but the olfactory portal seemed definitely to be ruled out.

H21. On Sept. 19, 1940, J. C., an eleven year old boy, complained of nausea, headache and general malaise.

Sept. 20. Severe pains in back and neck. CSF 220 cells per cu. mm., positive globulin test.

Sept. 21. Admitted to University Hospital, Ann Arbor, Michigan. Temperature was 102.4°; respiration, 24; pulse, 100. There was marked weakness in all extremities and breathing was mainly diaphragmatic. During the next four days breathing became more rapid (36 per min.). A chest respirator was ineffective because of its slow rhythm and intranasal oxygen was of no avail.

Sept. 25. Died. The brain was fixed in formalin, and was complete except for the olfactory bulbs.

Summary of microscopic findings: The distribution of lesions in the brain of this case (Table I) was quite similar to that observed in case H14, and suggests that the virus invaded the brain in an ascending direction from the spinal cord.

RESUMÉ OF PATHOLOGICAL FINDINGS

As has been mentioned in previous publications, the total pattern of distribution of lesions in brains of rhesus monkeys and of chimpanzees, after inoculation of virus by various portals, can give critical evidence regarding some of these portals even in late stages of the acute disease, or in fatal cases. Furthermore, it has been shown that the distribution of lesions depends not only on the path of ingress of virus into the brain, but on the differential susceptibilities of certain centers and their relative accessibility, in terms of nerve fiber connections, to infective concentrations of virus.

As early as 1907 Harbitz and Scheel described the distribution of lesions in the brains of seventeen cases of human poliomyelitis, and noted the absence of lesions in the olfactory bulbs and tracts. This finding was not taken seriously in this country until recent years, especially since these authors interpreted their findings as evidence in favor of a hematogenous portal of entry of the virus. The distribution of lesions in the brain as described by these authors, as reported also in greater detail by Környey ('33), by Horanyi-Hechst ('35), by Swan ('39), and by others, follows in general the pattern observed by us in the monkey and in the chimpanzee. So close is the resemblance in some respects that one is inevitably led to the conclusion that the factors involved in the spread of virus in the brain and in the selective injury of certain centers is common to all of these primates. It should be possible therefore to determine, by comparison with the

mass of experimental evidence available, the portal of entry in favorable human cases by the differences or similarities in the pattern with respect to those described in the experimental animal after inoculation by various portals. With regard to the olfactory portal it is clear that in the experimental animal lesions are found in the olfactory bulb and secondary olfactory centers only after inoculation by the intranasal route. In our material, however, and as noted by Swan ('39), it is precisely in these centers that lesions are most rarely found in human cases, so that grave doubt is cast on the importance of the olfactory route as a portal of entry of virus in man. The fact that in rare cases lesions are found in the olfactory bulbs and other olfactory centers (Table I) suggests that these centers are just as susceptible to pathological changes in man as in the lower primates, but, as in these experimental animals, the olfactory centers are spared only when the virus gains entry by some other portal. In several of our cases, the pathological process was so severe in the brain-stem centers caudal to the olfactory centers that it seems difficult to believe that the virus could have passed through the olfactory centers without leaving a trace. When the pathological process is equally severe in either the monkey or the chimpanzee after intranasal inoculation, the olfactory centers, including the olfactory bulbs, invariably show severe damage (Table I, A83).

It is thus apparent that not only the absence of lesions in the olfactory bulbs, as described by many workers for most cases, but also the characteristic distribution of the encephalitic lesions, tends to rule out the olfactory portal in man except in rare instances. The distribution of lesions in the brains of most of the cases described here unfortunately does not point precisely to the actual portal of ingress of virus, because in all of our cases the development of the pathological process in the brain is practically complete. Chance cases obtained in the pre-paralytic stage, early paralytic stage, or in a stage of early arrest, should give valuable information in this regard, but as far as we are aware such cases have not as yet been described. By analogy with the chimpanzee, which appears to react to poliomyelitis virus in a fashion practically identical with that seen in man, one can merely say that the virus in man probably gains access to the nervous system from the oral cavity, pharynx, or intestinal tract. After entering the

central nervous system via the spinal or cranial nerves, the virus ascends in the brain-stem in a fashion illustrated diagrammatically in Chart I. The distribution of lesions, and presumably of virus, in final stages of development of the pathological process is, in most cases, practically identical with that seen in the experimental animal after inoculation by non-olfactory peripheral portals (Bodian and Howe, '40, Howe and Bodian, '41b).

It is interesting that the description of the distribution of lesions in the human brain applies equally well to cases described from several different epidemics, over many years, and in far distant lands. This characteristic distribution, more than any other pathological feature of the disease, is probably pathognomonic of poliomyelitis as a disease entity in man, as it is in the monkey. The remarkable selectivity of the pathological process for certain centers has been noted by many investigators, and some of the factors involved in this selectivity have already been discussed in connection with experimental studies. Perhaps an even greater selectivity for the motor cortex occurs in man than in the monkey, since it is rare to find lesions in the human cortex outside of area 4 of Brodmann (André-Thomas and Lhermitte, '29; Spielmeyer, '32; Stiefler and Schenk, '33; Környey, '33b; Horanyi-Hechst, '35). Of 24 cases, Horanyi-Hechst found cortical lesions in area 4 in 19 cases; three cases also had lesions in area 6, and 2 cases had lesions in area 1. These areas are contiguous and are the regions of the cortex from which movements of skeletal musculature are most readily elicited by electrical stimulation. In our own series of thirteen cases twelve showed lesions in area 4, consisting of perivascular cuffing, neuronophagia, and focal mesodermal-glial infiltrations in all layers, but especially in the layer of Betz cells, two showed a few lesions in area 6, four showed a few lesions in area 1, and two contained very infrequent and light lesions in the frontal granular cortex, in one case as far rostral as the orbital gyri. No case was noted with a single lesion in the posterior parietal, occipital or temporal neocortex, or in the hippocampus or pyriform cortex although Swan ('39) has described one case with lesions in Ammon's horn.

Other characteristic features of the distribution of the pathological process are the apparently absolute refractoriness of the lateral geniculate body and visual cortex, the relatively high resistance of the

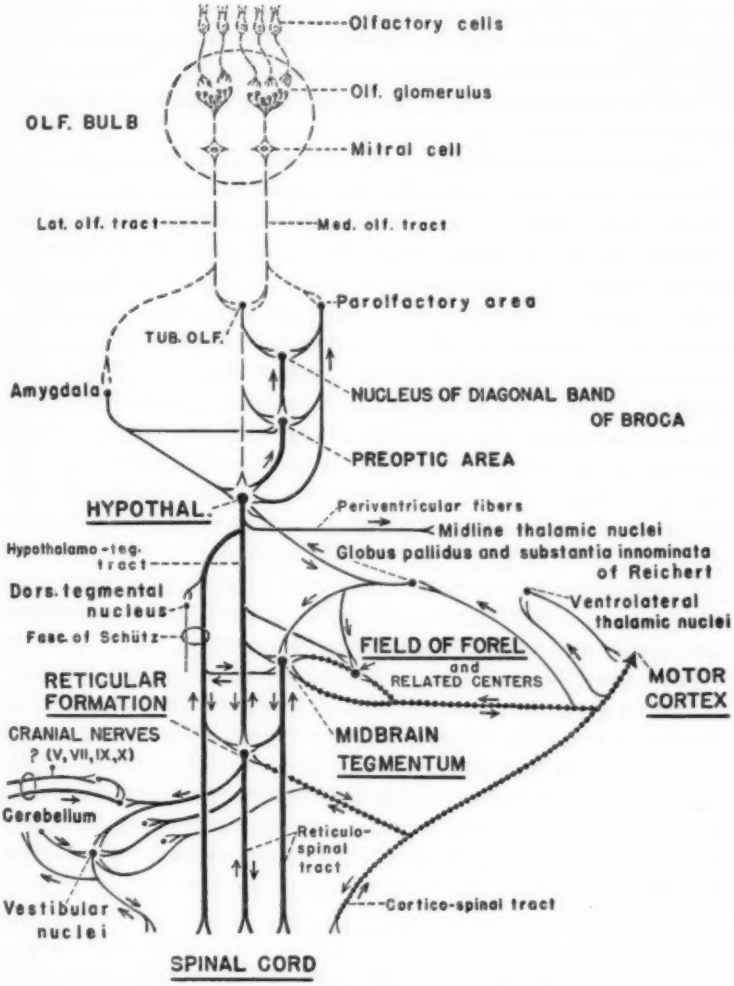


CHART I

caudate nucleus and putamen, the rare or extremely light involvement of the olfactory centers, the nucleus of Luys, the pontine nuclei,

the inferior olivary nucleus, and the cerebellar cortex (except that of the vermis), and the high susceptibility of the vestibular nuclei and associated cerebellar centers, especially the roof nuclei. These features are the same in the experimental animal which has been inoculated by non-nasal routes.

From the pathological point of view the elaborate classification of clinical types of the disease has little significance, except to emphasize a special degree of reaction of certain tissues or centers in the nervous system. It seems likely that in all cases of poliomyelitis except possibly in early cases of spontaneous arrest, an encephalitis exists, whether symptoms are present or not. This encephalitis, as well as a meningitis, may be of greater or less severity, but the distribution of lesions in susceptible centers varies but little. It is doubtful whether the localization of the initial paralysis is of any significance in indicating the region of ingress of virus into the central nervous system, except possibly in cases of *initial bulbar paralysis* (see also Swan, '39). By analogy with the chimpanzee (p. 149, this *Bulletin*), such cases would seem to suggest entry of the virus by way of the oral-pharyngeal portal, probably via the trigeminal, glossopharyngeal or vagus nerves. While the significance of initial bulbar signs in terms of localization of the portal of entry is most suggestive, their absence does not necessarily rule out the cranial nerves as routes of virus dissemination, since it is obvious that in many cases the virus, in passing along the cerebrospinal axis, whether brain stem or cord, may by-pass highly susceptible motor centers in one region, only seriously to affect similar centers at more rostral or caudal levels. This type of sparing of susceptible centers can probably be attributed, as can the process of arrest in general, to constitutional factors inherent in the host. As to the nature of these factors we are as yet quite ignorant.

DISCUSSION

The crux of the portal of entry problem in human poliomyelitis is the identification of the tissues through which an infective quantity of virus must pass to reach the nervous system. Under reasonably favorable experimental conditions this can be done with considerable accuracy by a search for either lesions or virus during the early stages

of the disease. In later phases both of these methods of investigation suffer from the same limitation, the fact that virus has achieved a wide dissemination throughout the body. It therefore becomes necessary to emphasize the findings derived from tissues in which the conditions determining the spread and localization of the virus are relatively well understood. For example, it has not been possible as yet, with the histological methods available, to determine even the presence of virus in the gut or other viscera, not to speak of the precise cellular localization or mode of spread of virus in these tissues. Such information regarding differential cell susceptibilities, and differential localization of the virus in either nerve cells, epithelial cells, or cells derived from the mesoderm in the viscera is essential for a valid interpretation of the mode of spread of virus from such peripheral tissues to the central nervous system. Furthermore, the method of virus assay of these tissues in man suffers from the grave limitation that it ascertains only the distribution of virus at the time of death, usually late in the course of the disease, in regions where cellular localization is again impossible. The nervous system offers the best opportunity for the analysis of the distribution of lesions in the late stages of poliomyelitis for the reason that the conditions of axonal spread are relatively well understood and the virus leaves a histologically demonstrable trail which persists for many days. In the nervous tissues there is close agreement between our results and those obtained by the virus assay studies of Sabin and Ward ('41), who worked with similar material obtained, for the most part, at the same times and in the same epidemic areas (*i.e.*, Northern Indiana and West Virginia in the late summer of 1940). These observers found the olfactory bulbs, nasal mucosa and the anterior portions of the brain stem (preoptic area and olfactory trigone) negative, although virus could be demonstrated with ease in the spinal cord, medulla, midbrain, thalamus and motor cortex. It is also worthy of note that the frontal and occipital cortex was negative for virus. The method of virus assay thus appears to agree quite closely with the histological method in indicating the distribution of virus in the human brain. The chief limitations of the former grow out of the difficulties of fine localization and the fact that virus titre drops rapidly after a certain time, so that accurate observations

cannot be made late in the disease. On the whole, however, the agreement of findings with the two methods is excellent, both with respect to regions where invasion is usual, and with respect to regions where there is no evidence of invasion.

It seems quite definite that while the olfactory bulbs are susceptible to virus (Case H10), the olfactory portal is not the usual one. The anatomical findings in case H7, which showed bilateral necrosis of the nucleus ambiguus, are nearly identical, as regards this region, with the findings in case 9 of Sabin and Ward ('41). In the latter case there was also a primary bulbar paralysis showing heavy lesions in the nucleus ambiguus; this was undoubtedly poliomyelitis although no virus was recovered, presumably because of the relative chronicity of the disease before death. Such cases considered in the light of the recognized association of bulbar poliomyelitis and tonsillectomy (Aycock and Luther, '29; Silverman, '31; Stillerman and Fischer, '38; Koskoff, Amshel and Lebeau, '39; Fischer, Stillerman, and Marks, '41, etc.) indicate that the nerves supplying the pharynx probably serve as one pathway to the CNS. In this connection the fact that the pharynx is one of the principal sites in which virus is found outside the nervous system (Sabin and Ward, '41) may be significant. Although the 5th nerve was implicated in the chimpanzee, the human material was not suitable for the determination of the role of this nerve in man.

As regards the gastro-intestinal tract as a portal of entry, the evidence is not as clear as could be desired. Reasoning by analogy from the monkey and chimpanzee one can only state that the pathological picture in some of the brains strongly resembles that of an infection ascending from the spinal cord, but from a pathological point of view it is not possible to determine the precise pathway followed from the periphery, since the findings do not exclude the cutaneous nerves as conveyors of virus. The presence of virus in the feces and colonic contents, the contents and wall of the ileum (older findings summarized by Vignec, Paul and Trask, '38: recent confirmations by Lepine, Sedallian and Sautter, '39; Kling, Olin, Magnusson and Gard, '39; Kramer, Gilliam and Molner, '39; Trask, Paul and Vignec, '40; Howe and Bodian, '40; Sabin and Ward, '41) points to the alimentary tract as a region of virus proliferation, but does not necessarily prove that it is an immediate portal of entry into the

nervous system. The isolation of virus from the coeliac ganglion in one case (Sabin and Ward, '41) strongly suggests the sympathetic nerves as a route for infection but is not entirely conclusive since lesions in the coeliac ganglia of chimpanzees are correlated with the presence of virus in the stools (Howe and Bodian, '41b) and have been found in instances where the primary route of infection is known to have been olfactory. Nevertheless the anatomical evidence favors the possibility that in some instances the virus travels from the gut to the spinal cord over sympathetic nerves, although it also indicates the participation of the vagus nerve in the spread of infection from this area to the medulla. Case H18, a fulminating primary bulbar case, showed unusually heavy lesions in the vagal nuclei rather than in the nucleus ambiguus, which suggests the gut and not the pharynx as the initial point of virus penetration.

Certainly the finding of virus and lesions in the coeliac ganglia of the chimpanzee and man does indicate that in these higher forms the virus is also strongly neurotropic in the peripheral nervous system and that its occasional and sometimes questionable isolation from mesenteric (Kling, Olin and Gard, '38) and other lymph nodes (Sabin and Ward, 41) is possibly not of great significance. However as Theiler ('41) has pointed out, evidence for strict neurotropism of the virus has in the past been derived entirely from an unnatural host, the rhesus monkey. The absence of virus in the alimentary tract of this animal and its presence in the chimpanzee and man indicates a change in the virus-host relationship which may mean an increase in the potentialities of the virus for interaction with non-nervous tissues. Too little is known of the locus of proliferation of virus found in the alimentary tract to settle this problem Theiler ('41) has recently drawn some strong parallels between spontaneous mouse encephalitis and human poliomyelitis, but it is interesting in this connection that Olitsky and Schlesinger ('41) were unable to determine the mode by which this virus enters the nervous system under natural conditions, although it is known to be present in the intestinal tract of nearly every laboratory mouse. This was also the case, however, even when the disease was artificially produced; for example, lesions were not found in the olfactory bulbs of animals succumbing to intranasal inoculation. The virus of mouse encephalitis has been too

little studied as yet to make possible the interpretation of such facts or to determine whether they argue for or against neuronal transmission from the periphery in this animal. While poliomyelitis virus has been shown to present a remarkably constant tendency to follow nerve pathways even in different primate hosts, one must not be blind to the possibility that there are certain species differences which may not yet be fully appreciated in relation to a natural host.

SUMMARY

1. The distribution of lesions in the human brain in poliomyelitis is entirely comparable with that seen in the rhesus monkey and the chimpanzee, when these animals are inoculated by non-nasal portals. This indicates that dissemination of virus in the central nervous system follows fiber pathways in the same fashion demonstrated for these other species, and that their differential cell susceptibilities within the brain and spinal cord are the same. From the histopathological point of view the elaborate classification of clinical types of the disease has little significance, except to emphasize a special degree of reaction of certain tissues or centers in the nervous system.

2. Histological findings show that human olfactory bulbs are capable of reacting to virus, but indicate that the olfactory portal was not operative in any of the cases studied.

3. Many facts point to the alimentary tract as a locus of virus proliferation, and in many instances the material suggests the spinal cord as the primary site of invasion of the central nervous system. It has not been possible however to determine the precise pathway of the virus from the gut to the central nervous system, except in one case where the vagus nerve was strongly suggested.

4. Evidence is adduced which suggests that the nerves supplying the pharynx were the path of entry in at least one case.

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PLATE I

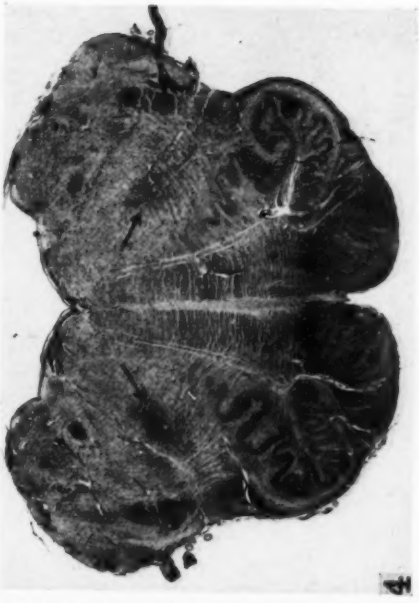
FOUR LEVELS OF THE BRAIN FROM CASE H7

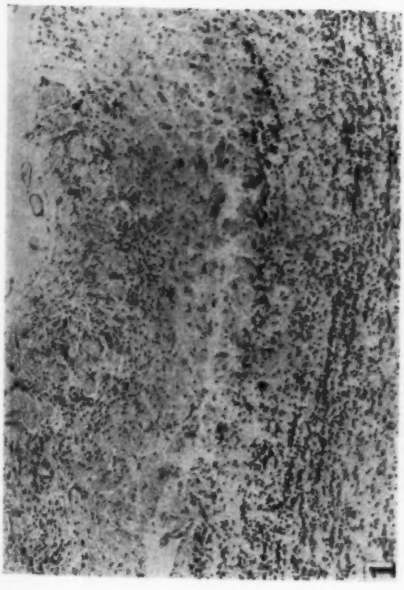
FIG. 1. Olfactory bulb showing normal configuration. ×60.

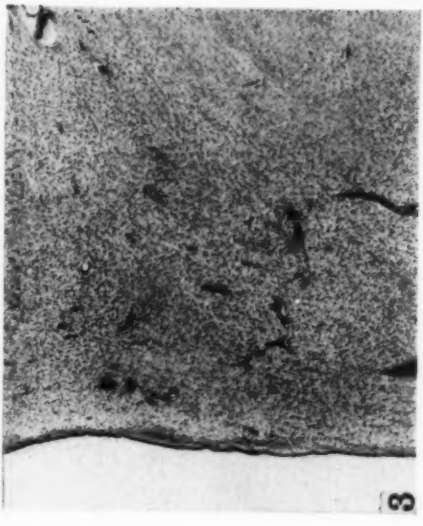
FIG. 2. Right anterior olfactory nucleus—no lesions. ×20.

FIG. 3. Right hypothalamus. Note perivascular cuffing and mesodermal-glial foci. ×20.

FIG. 4. Medulla. Note the extensive invasion of the nucleus ambiguus on both sides (arrows). ×5.







FIGS. 1–4

PLATE II

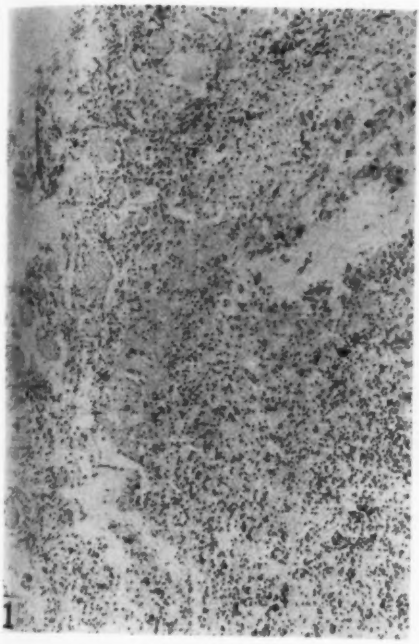
CASE H9. (DIED OF ACUTE APPENDICITIS)

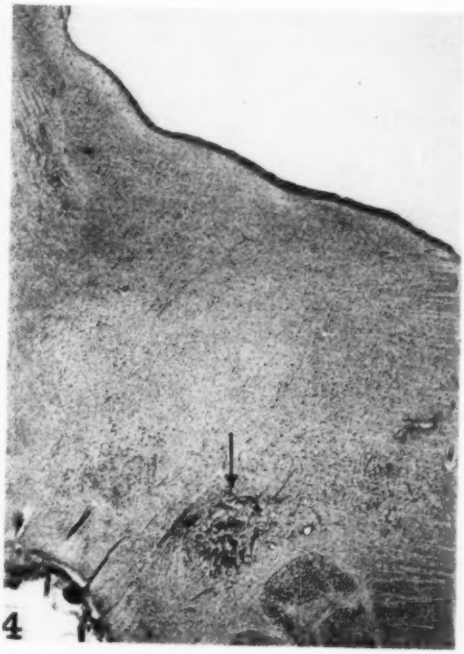
FIG. 1. Olfactory bulb, ×60. There is considerable post mortem autolysis but no lesions of poliomyelitis.

FIG. 2. Right anterior olfactory nucleus. No lesions. ×20.

FIG. 3. Right hypothalamus at the level of the paraventricular nucleus. ×20. Note fiber bundles of the fornix at top of picture. There are a few lightly cuffed vessels at this level.

FIG. 4. Left medulla. ×5. Note the large area of neuronophagia in the reticular formation between the facial nucleus and the inferior olive, and lesions in vestibular nucleus (upper left).





FIGS. 1–4

PLATE III

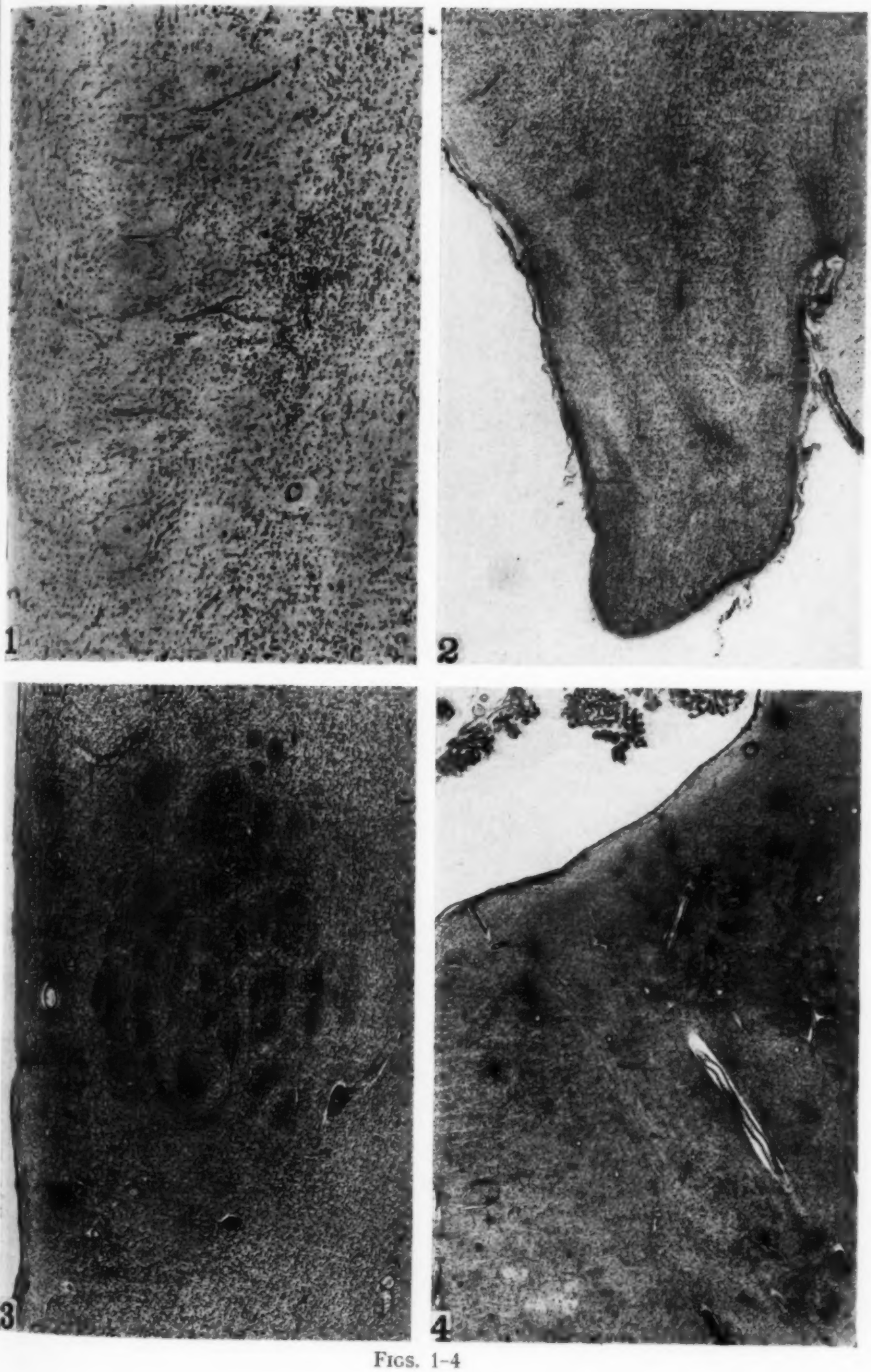
CASE H10

FIG. 1. Left olfactory bulb showing cuffed vessels and area of neuronophagia in the mitral cell layer. ×57±.

FIG. 2. Left anterior olfactory nucleus showing absence of lesions. ×20.

FIG. 3. Left hypothalamus at level of paraventricular nucleus and fornix. Note perivascular cuffing and occasional mesodermal-glial foci. ×20.

FIG. 4. Right medulla. ×5. Note neuronophagia in ventral part of facial nucleus (lower right), vestibular nuclei (upper right), and reticular formation (upper left).



FIGS. 1–4

PLATE IV

Case H12. (Most Severe of Series)

Fig. 1. Olfactory bulb, ×60, showing single cuffed vessel in the white matter which is thought to represent extension of reaction out from the balance of the brain.

Fig. 2. Left anterior olfactory nucleus. No lesions. ×20.

Fig. 3. Right hypothalamus at the level of the paraventricular nucleus and fornix. Note extensive reaction. ×20.

Fig. 4. Right medulla, ×5, showing neuronophagia in the facial nucleus, vestibular nuclei and reticular formation.

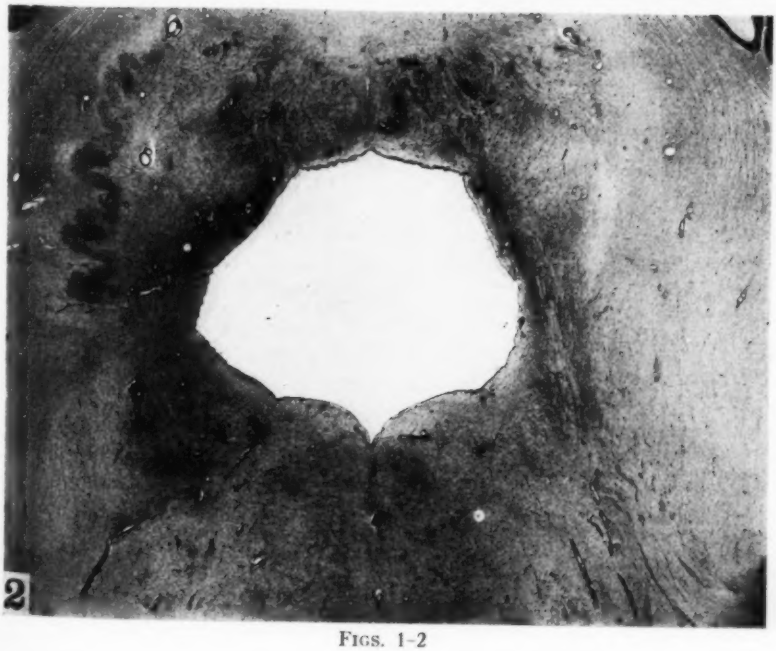
FIGS. 1–4

PLATE V

CASE H12

FIG. 1. Left cerebral cortex showing the central sulcus (center). Note lesions in precentral (motor) cortex and their absence from post central cortex. ×5.

FIG. 2. Dorsal portion of medulla, ×5, showing the extension of lesions from the vestibular nuclei to the roof nuclei of the cerebellum.



FIGS. 1–2

PLATE VI

Case H12

Fig. 1. Left amygdala, ×5, showing absence of lesions. Note focus in the substantia innominata of Reichert (arrow).

Fig. 2. Right hippocampus, ×5, showing absence of lesions. Note invasion of the substantia nigra (arrow).

FIGS. 1–2

A NEW TREATMENT FOR BURNS

Preliminary Report

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Submitted for publication June 25, 1941

It has long been realized that infection is a serious complication of burns and various methods of preventive treatment have been tried, among them being the local application of tannic acid which was recommended by Davidson (1) in 1925, and this method with various modifications has had wide acceptance. In 1933 gentian violet was introduced by Aldrich (2) who later found that a combination of gentian violet, brilliant green, and neutral acriflavine offered all of the better qualities of gentian violet in the specificity against the gram-positive organisms, plus an equal specificity against the gram-negative ones (3).

In view of the great effectiveness of the newer chemotherapeutic agents in combating infection, it occurred to the writer that these might be used advantageously to combat infection in patients with burns. Last January, therefore, several burns were treated in the manner to be described below with results which were so encouraging that this method has been applied routinely to the treatment of all burns that have since come to this hospital. This method of treatment has been found to be much superior to any of the other methods of treatment previously used here.

During this six months' period, fifteen patients have had burns and scalds severe enough to necessitate hospitalization, and one hundred patients have been treated in the surgical outpatient department.

When a patient with a burn extensive enough to warrant hospitalization enters the accident room, he is placed at once on sterile sheets. Regardless of whether shock is already present or not, measures are immediately instituted to combat it since the general treatment of the patient is our primary concern. Cultures are taken from various

burned areas. While the accident room surgeon is scrubbing, the attending nurse sprays the burned areas with 3 per cent sulfadiazine (2-sulfanilamidopyrimidine) in 8 per cent triethanolamine, using an atomizer. This treatment allays a great deal of the patient's pain, and a narcotic may not be necessary. The burned areas are not washed, but simply sprayed, and it is quite unnecessary to clean the burned areas with any other agent. Scrupulous care and aseptic technic are exercised in the careful débridement of the blebs and loose tissue.

The patient's bed is brought to the accident room, transfer to and from a stretcher thus being avoided The bed linen is sterile. A fracture bed is useful when it is desirable to suspend an extremity, and the use of the bed pan is thereby greatly simplified. A heat cradle with blue bulbs to prevent glare is used to keep the patient warm. The temperature of the cradle should not exceed 90°F.

The burned areas are sprayed every hour during the first day, every two hours the second day, every three hours the third day, and every four hours the fourth day. By this time a thin, translucent eschar has formed, and further spraying is not essential. Through the translucent eschar thus formed, the progress of the healing can be followed, and in second degree burns the regeneration of the epithelium can be observed. The eschar is sufficiently strong and yet pliable and elastic enough to prevent breaking, and active exercise and partial weight bearing are encouraged to prevent contractures.

After about ten days the edges of the eschar begin to loosen and to separate from the intact epithelium beneath it. At this time compresses of the sulfadiazine-triethanolamine mixture can be applied; in many instances sterile mineral oil sprays followed by normal saline compresses allow the eschar to be removed in large sheets. In third degree burns the eschar is allowed to remain in situ for at least two weeks.

Patients having burns of second degree severity, and involving not more than twenty per cent of the body surface are not hospitalized. In these patients débridement of the second degree burns is carried out by the same technic that is employed in the more extensive ones. They are observed in the accident room for at least two hours during which time the burned areas are frequently sprayed with the sulfa-

diazine-triethanolamine solution. Before the patients are discharged the burned areas are covered with either sterile vasaline gauze or an ointment consisting of 5 per cent sulfadiazine and 8 per cent triethanolamine in a stearin base. No eschar forms unless the burned area is exposed as in the case of the hospitalized patients. These patients return in twenty-four or forty-eight hours. Cultures are taken from the burned areas, after which they are again sprayed several times and then dressed with either the ointment or the vasaline gauze. The same procedure is carried out on return visits until the burned areas are healed.

The sulfadiazine solution has a pH of about 8.7. It is clear and has a faint yellow color, which may darken slightly, owing to the oxidation of the sulfadiazine, unless the solution is stored in dark bottles. It does not stain. It is almost odorless and has a bitter taste. It does not injure skin, mucous membrane or granulating surfaces, and can be used in and around the eyes with impunity. It is a powerful penetrant and can be detected in the blood within several hours after having been sprayed on a burned surface. For this reason sulfadiazine blood levels were determined daily for several days in patients receiving multiple daily treatments. When the eschar has formed, the blood level rapidly approaches a minimum even though the spraying is continued. If then desired, the drug may be administered orally. We feel confident that regardless of how often the spray is applied, it does not coagulate or destroy normal tissue.

The results obtained in one hundred and fifteen burned patients have been gratifying. It is the general opinion of the Surgical Staff that the burned areas have healed more rapidly than with any form of treatment previously used at the Johns Hopkins Hospital. No infection has occurred in any of the cases treated in the outpatient department. In only two of the fifteen more extensive cases admitted to the hospital was there any evidence of infection under the eschar. In all these cases cultures were made from the surface of the burn after having removed aseptically a window of eschar. Only in the two cases mentioned have the cultures shown any growth of bacteria. In these two instances infection occurred while the eschar was being compressed preparatory to removal.

As regards toxicity, it may be stated that in only one case out of one

hundred and fifteen have any toxic effects been observed. A four-year-old child with second and third degree burns involving more than 80 per cent of the body surface was treated with the sulfadiazine-triethanolamine solution. The child entered the accident room *in extremis*, and succumbed within forty-eight hours. Only parts of the feet and ankles were not burned, consequently no blood samples could be obtained. Pathologic sections disclosed marked vacuolization of the cells of the kidneys and liver. In order to determine, therefore, whether the triethanolamine is toxic, dogs weighing 10 kg. were given 100 cc. of an 8 per cent solution of triethanolamine intravenously daily for one week without clinical effect and without demonstrable histologic lesions. Rabbits showed no lesions after having received 50 cc. daily by stomach tube for one month, or when 25 cc. were administered intravenously daily for one week. Guinea pigs which received 10 cc. twice daily by stomach tube for one week and mice which received 1 cc. twice daily by gavage for one month showed no lesions. Rats given 10 cc. twice daily by stomach tube for one week developed necrosis of the renal epithelium with regeneration as evidenced by mitosis. The sulfadiazine mixture when given to animals in large doses is definitely toxic and will cause specific lesions in the kidneys. As is known, sulfadiazine itself when administered in doses sufficient to produce high blood levels injures the kidney (4).

Because of the superior results obtained with this sulfadiazine-triethanolamine solution, and because of the absence of any indication of a toxic effect except in the single case of a very extensive burn mentioned above, we are continuing to use this method in the treatment of all burns at the Johns Hopkins Hospital. A full report will be published at a later date.

The sulfadiazine was kindly supplied by Dr. Perrin H. Long; it had been prepared by the American Cyanamid Company. Mr. Frank Reinish prepared the ointments and solutions.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Doctrina y Practica de la Profilaxis de la Tuberculosis. By LUIS SAYE. 355 pp. Illus. *Editorial Sudamericana, Buenos Aires* (1940?).

This book contains interesting and much-needed information relating to the epidemiology of tuberculosis in South America where, in large areas, the death rate from the disease is still deplorably high. In contrast to the marked and progressive decline in tuberculosis mortality in recent years in most countries of western civilization, the mortality in various parts of South America is increasing. There is reason to believe that this is due primarily to the fact that South America is now in a period of industrial expansion, concentration of population in industrial centers and increase in facilities for communication similar to that experienced by most other western countries a century ago. The ill-effects of industrialization in the period before its benefits reach the working classes and improve their living conditions are now familiar. The large Indian population, with a native resistance to tuberculosis apparently lower than that of whites, will, of course, accentuate the adverse influence of the early phase of industrialization in South America.

The author presents the results of x-ray and tuberculin surveys, and also his experience with prophylactic BCG vaccination, from which he concludes that a great deal of good can be accomplished by means of it. The difficulties in evaluating the results of prophylactic vaccination against tuberculosis in man are well known. The present study can be regarded only as a further addition to the ever-growing body of suggestive evidence that vaccination increases the resistance of the human being to tuberculosis, as it clearly does in the case of experimental animals.

The author's clinical observations on vaccinated children lead him to agree with the experimental demonstration that acquired resistance in tuberculosis is not dependent upon hypersensitivity.

The frequency with which tubercle bacilli can be found in the sputum and gastric contents of individuals who have asymptomatic lesions is stressed. In the author's experience, bacilli could be demonstrated by careful and repeated examinations in over 80 per cent of individuals with asymptomatic primary lesions. This recalls Stiehm's demonstration of tubercle bacilli in the gastric contents of 71 per cent of 21 persons with asymptomatic, "minimal" x-ray shadows. The frequency with which such "carriers" exist in the population, and the extent to

which they may serve to spread the infection, are important matters for study in all localities.

A. R. R.

A Topographic Atlas for X-Ray Therapy. By IRA I. KAPLAN and SIDNEY RUBENFELD. 120 pp. 55 plates. $4.00. *Year Book Publishers, Chicago, 1939.*

This extremely simplified collection of anatomical charts should prove to be of value as a handy reference to technicians in those X-Ray departments in which it is impossible for a physician to set up every treatment field at every visit. Needless to say, it can not and should not be expected to supplant the function of a trained Radiologist.

W. H. B.

Lectures on Conditioned Reflexes. Volume 2. Conditioned Reflexes and Psychiatry By IVAN PETROVITCH PAVLOV. *Translated and edited by W. Horsley Gantt.* 199 pp. Illus. $4.00. *International Publishers, New York, 1941.*

This is volume two of Pavlov's lectures on conditioned reflexes and concerns itself with that period of Pavlov's work from 1928 to his death in 1936 and with "a trial excursion of a physiologist in the field of psychiatry." There are sixteen separate articles collected from various sources besides a number of appendices included in the volume, and a number of items of interest to psychiatrists and psychologists are brought under Pavlov's scrutiny; for example, experimental neuroses, the hypnotic state, physiological attempts at explanation of hysteria, similar attempts at the explanation of obsessions and paranoia, and so on.

The translator has done an excellent job in several respects: he has brought together some of the productions of Pavlov's last years into a volume continuous with Pavlov's first volume of lectures on conditioned reflex; he has made the English quite readable and forceful; and in his introduction to the text, he gives a telling portrait of Pavlov in his last years, at the same time offering some sound criticism of Pavlov's attempt at physiological explanation of psychology.

It is asking too much to give in brief a telling critical statement of the value of Pavlov's work for psychology and psychiatry. Such a statement would require a book fully as large as this one, and it still needs to be done. As with many other important movements in our field, conditioned reflex ideology has suffered from overenthusiasm on the one hand and nihilistic rejection on the other. For purposes of this review it will suffice to say that Pavlov's resorting to analogy to bridge the gap between what he calls physiological states in animals and special states of the human organism which fall within the province of psychiatry leaves very much to be desired. This reviewer sees only analogy and not explanation in this sort of treatment of the material. Not that it is not without interest and suggestive of further research which ought to be done; and I am sure that this is exactly what Pavlov would have wished to be the fate of his contribution, namely, an incentive to further effort.

The chapter entitled *Reply of a Physiologist to Psychologists* (which by the way

was translated by Richard S. Lyman), is especially interesting and a forceful statement of Pavlov's views as opposed to those of some more traditional schools of thought in psychology. About the only common meeting ground that Pavlov would have with the psychologists whom he is contradicting, would be the demand for more and more telling experiment.

This book along with its predecessor is required reading for anyone doing experimental work in psychology and in "physiology of the higher nervous activity."

W. M.

Introduction to Psychobiology and Psychiatry. A Textbook for Nurses. By ESTHER LORING RICHARDS. 357 pp. $2.50. *The C. V. Mosby Company, St. Louis, 1941.*

This little book contains Dr. Richards' lectures to the nurses in the Johns Hopkins Hospital Training School for Nurses. The book embodies years of effective teaching of nurses who long ago came to value her services. It tells in a direct, forceful manner what nurses need to know about personality study and the elements of psychiatry to make their practical work best understandable. It follows rather closely Dr. Meyer's teaching and will no doubt be found useful by nurses everywhere. Those who have had occasion to hear Dr. Richards' lectures will be glad that the text preserves the forcefulness of her own personality.

W. M.